A Method for the Introduction of Reporter Groups into Moenomycin A, Based on Thiouronium Salt Chemistry

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p-Alkoxy-substituted cinnamylthiouronium salts **7**, **13**, **21a**, and **21b** reacted selectively with the 2-acylamino-1,3-cyclopentanedione unit A of moenomycin A (**1**) to give the corresponding 2-alkylated products **14a**, **14b**, **22a**, and **22b**. These products, depending on the pH of the solution, were either stable under the reaction conditions or they underwent retro-Claisen-type reactions. The method can be used for the attachment of reporter groups to moenomycin A for the synthesis of, for example, **25**.

Introduction

The shape of bacteria is determined by a net-like multilayer polymer surrounding the cell. The polymer − peptidoglycan − consists of repeating β-1,4-linked *N*-acetylglucosaminyl-*N*-acetylmuramyl units cross-linked by short peptide side chains.[1] The biosynthesis of peptidoglycan is an essential pathway for bacteria and has no direct counterpart in eukaryotic cells. Defects or disruption of peptidoglycan or inhibition of its biosynthesis result in cell lysis caused by the osmotic pressure. The distinct stages of peptidoglycan biosynthesis offer attractive targets for the development of selective antibacterial agents.

The biosynthesis of peptidoglycan in *E. coli*, starting from a membrane-bound undecaprenyl-linked disaccharide precursor (lipid II), is completed by two successive reactions, a transglycosylation reaction producing unbranched glycan strands[2] and a transpeptidation reaction cross-linking the peptide units of different strands.[1] Both reactions are catalyzed by the major high molecular weight *penicillin-binding proteins* (PBPs).[3] PBPs such as PBP1a and PBP1b are bifunctional enzymes with two separate active sites, one for transglycosylation and the other for transpeptidation. Each of these domains can be specifically inhibited by antibiotics. While β-lactam antibiotics exert their action through covalent binding to an essential serine residue in the transpeptidase domain, the transglycosylation step of cell wall assembly can be blocked by a number of antibiotics, including the moenomycins.[4] Of these, the moenomycins (see moenomycin A, **1**, Figure 1) are the only compounds known to inhibit the enzyme.[2]

The structure-activity relationships of the moenomycins have been studied extensively[5] and a mechanism for their mode of action has been proposed.[6,7] It is assumed that they are anchored to the cytoplasmic membrane through the C_{25} lipid component, followed by highly selective recognition of the oligosaccharide moiety at a substrate binding site of the enzyme, most probably the binding site of the growing glycan chain (the glycosyl donor). Whereas the mechanism of the transpeptidation reaction is reasonably well understood, the active site of the transglycosylase is still unknown and the mechanism of the transglycosylation step is largely unexplored. The moenomycins represent a unique tool for elucidating the structure of the enzyme and the detailed mechanism of the transglycosylation reaction. One array of methods that can be used for this purpose is based on the attachment of moenomycin to solid supports (affinity chromatography for isolating PBP1b[8] or moenomycin aptamers[9]), reporter groups (such as fluorescent labels for binding studies,[10,11] photoactive groups for affinity labeling,[12] etc.) or proteins (raising of antibodies[13]). For moenomycin A, there are a number of essentials that have to be taken into account:

(i) A selective reaction has to be used in order to be sure that a structurally well-defined conjugate is formed.

(ii) The conjugate must bind efficiently to the moenomycin binding site at the enzyme (it should be antibiotically active). This means that the conjugation reaction should not occur at units C-E-F-G-H-I (see **1**, Figure 1), because they have been shown to be prerequisites of antibiotic activity.[5]

(iii) The reaction has to be performed in highly polar media, preferably in aqueous solution, for solubility reasons.

Some time ago we reported that the enolized 1,3-diketone unit A of moenomycin A reacts selectively with *p*-acceptor-substituted aromatic diazonium salts − that is, with soft

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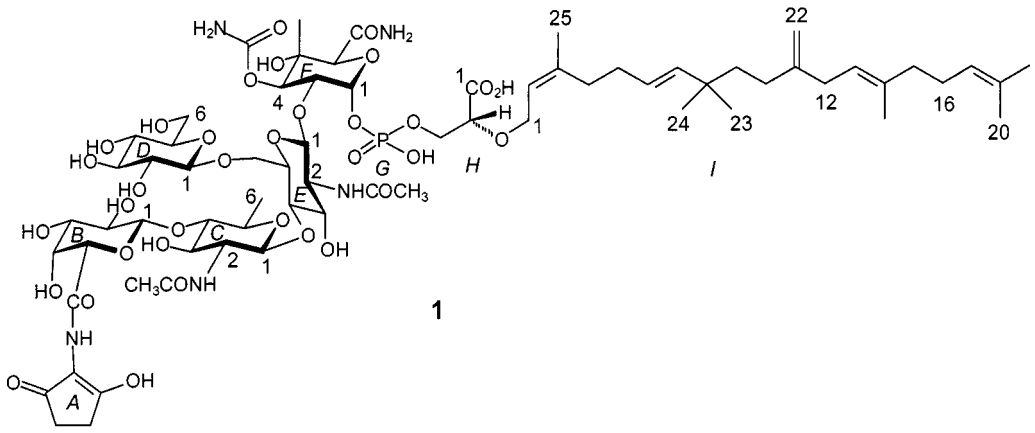
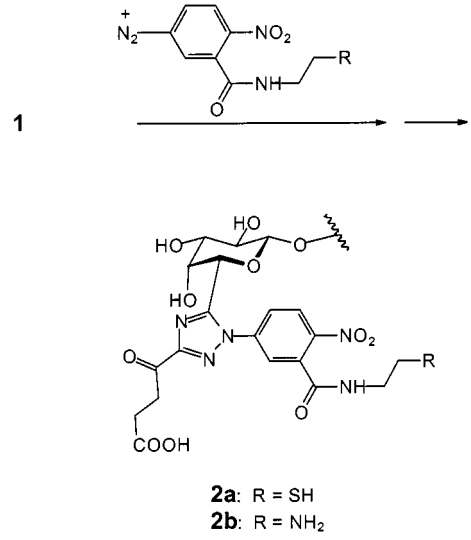


Figure 1. Moenomycin A

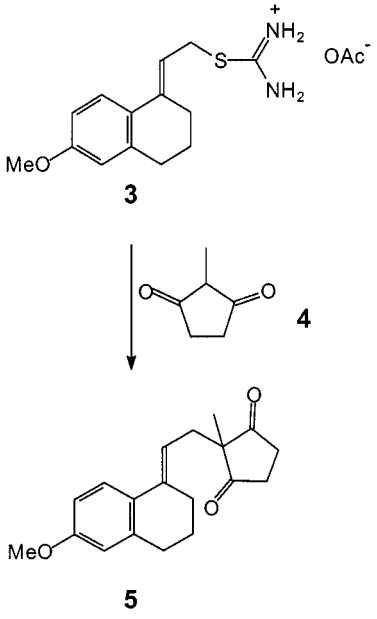
electrophiles – with a well-balanced reactivity (see Scheme 1) to give the corresponding azo derivatives. These were not stable under the reaction conditions, and underwent Japp–Klingemann cleavage to form the corresponding amidrazones, which slowly cyclized to yield triazoles.[14] We described the synthesis of some triazoles in which the functional groups R (SH, NH_2) were orthogonal in their reactivities to all other functional groups in **2a** or **2b** and could be used for a large variety of conjugation reactions.[8–10,12,13] Here we wish to outline experiments with another class of soft electrophiles, which eventually resulted in a new conjugation method.



Scheme 1

Results and Discussion

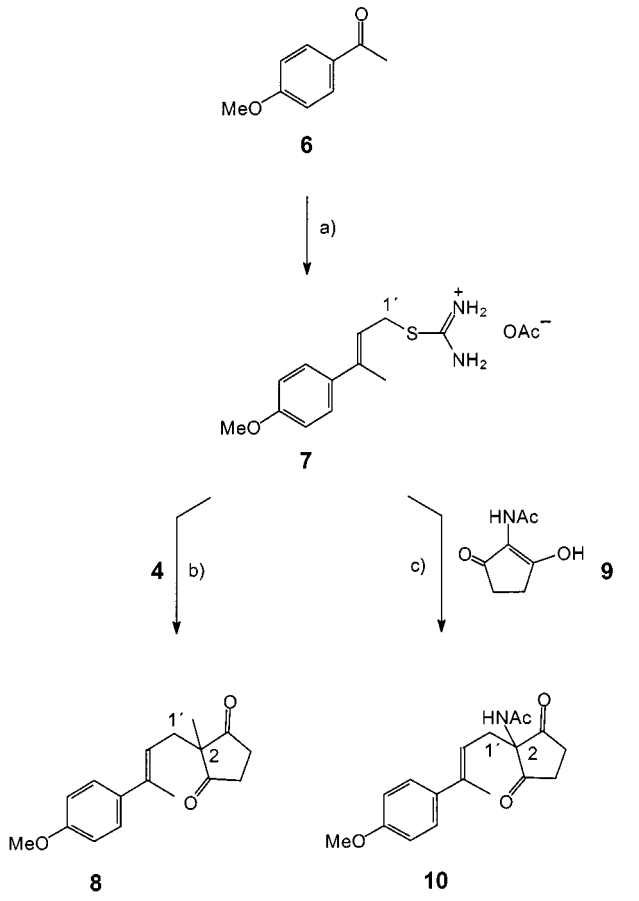
In the course of synthetic work in the steroid field, Kuo, Taub, and Wendler reported in 1965 that thiouronium salt **3**, on treatment with 2-methylcyclopentane-1,3-dione (**4**) in aqueous solution, provided secosteroid **5** in 80–85% yield (Scheme 2).[15] The method was later used by others.[16,17]



Scheme 2

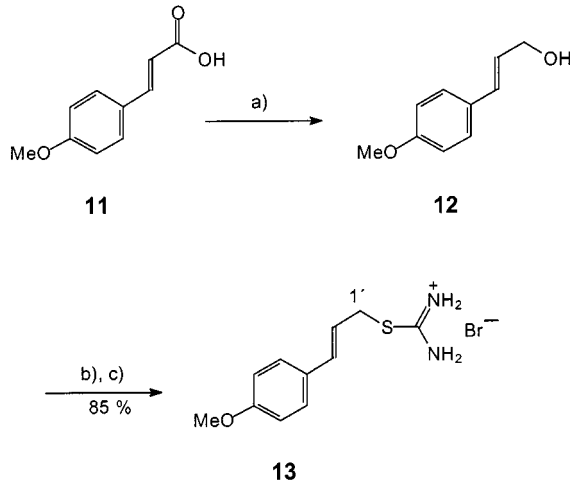
Repeating work by Schick and Hilgetag,[16] we obtained **8** from **4** and thiouronium acetate **7** in 80% yield. Under the same conditions, moenomycin model compound **9** was converted into alkylation product **10** in 71% yield. Thiouronium acetate **7** was prepared from *p*-methoxyacetophenone (**6**) (see Scheme 3) as reported previously.[16]

Since the olefinic methyl group (see Formula **7**) was not essential for our purposes, and since we had found in the alkylation experiments that, instead of the thiouronium acetates used in all previous reports, the corresponding bromides could also be employed, we prepared thiouronium bromide **13** from (*E*)-*p*-methoxycinnamic acid (**11**) as summarized in Scheme 4. The yields in the individual steps were very satisfying and in our hands appreciably higher than



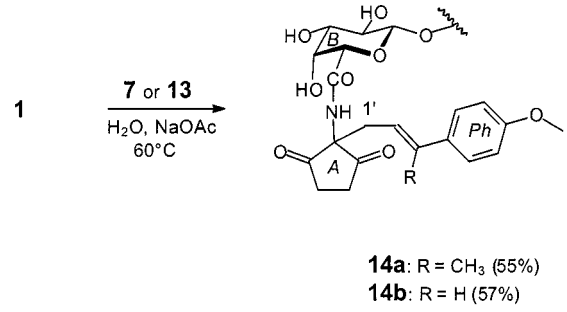
Scheme 3. a) refs.[16,19]; b) water/toluene, 60 °C, 80%; c) water/toluene, 60 °C, 71%

those found in the synthesis of **7**. Furthermore, bromide **13** crystallized very nicely from acetonitrile solution.



Scheme 4. a) refs.[18,19]; b) PBr_3, −10 °C; c) $SC(NH_2)_2$, acetonitrile

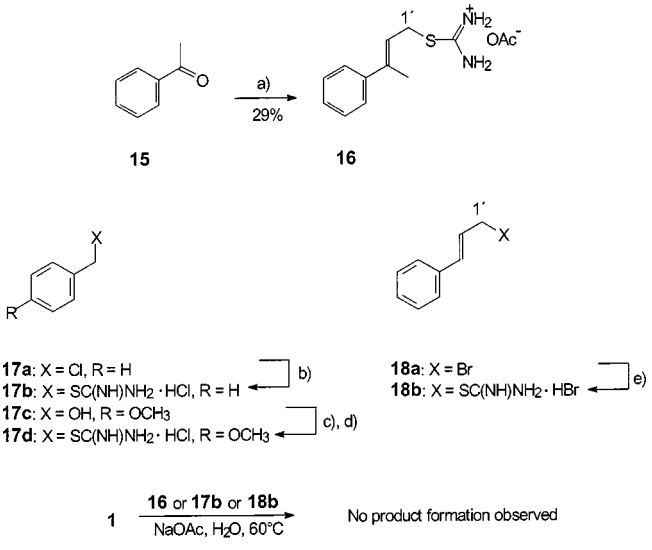
Compounds **7** and **13** both reacted readily with moenomycin A in aqueous solution buffered with sodium acetate to give alkylation products **14a** and **14b**, respectively, in reasonable yields (57% and 55%, Scheme 5).[20]



Scheme 5

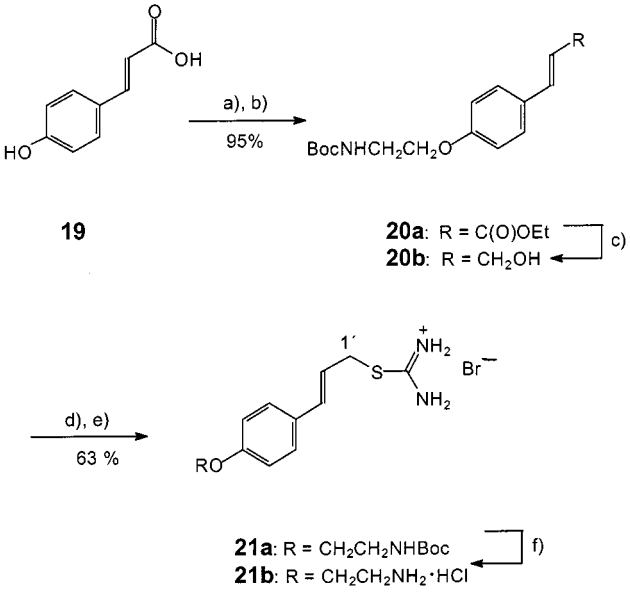
The structure of **14b** was determined with the aid of 2D NMR experiments. Thus, the diastereotopic aliphatic CH_2-1′ protons (broad multiplet at $\delta = 2.60-2.69$) were assigned by their correlation with 2′-H (^{1}H, ^{1}H COSY). The corresponding carbon C-1′ signal at $\delta = 36.89$ was assigned on the basis of long-range couplings with 2′-H and 3′-H (HMBC). The CH_2-1′ protons were found to have long-range couplings to C-1^A, C-2′, C-3′, and to the two carbonyl carbon atoms C-2^A and C-5^A. The ESI ICR, FAB, and MALDI TOF mass spectra displayed the expected molecular ion peaks. The structure of **14a** was accordingly deduced.

In order to find out ways in which the thiouronium salts could be modified to allow the attachment of reporter groups, we prepared thiouronium salts **16**, **17b**, and **18b** as depicted in Scheme 6. None of these salts reacted with moenomycin A in aqueous, acetate-buffered solution at 60 °C. The *p*-methoxy-substituted benzylthiouronium chloride **17d** did react with **1** to give the expected conjugate (formula not shown), but only very slowly (144 h at 75 °C) and with insufficient yields (7%). This implied that the conjugated olefinic double bond and also an alkoxy substituent in the aromatic ring should be present in thiouronium salt reagents suitable for conjugation reactions with **1**.



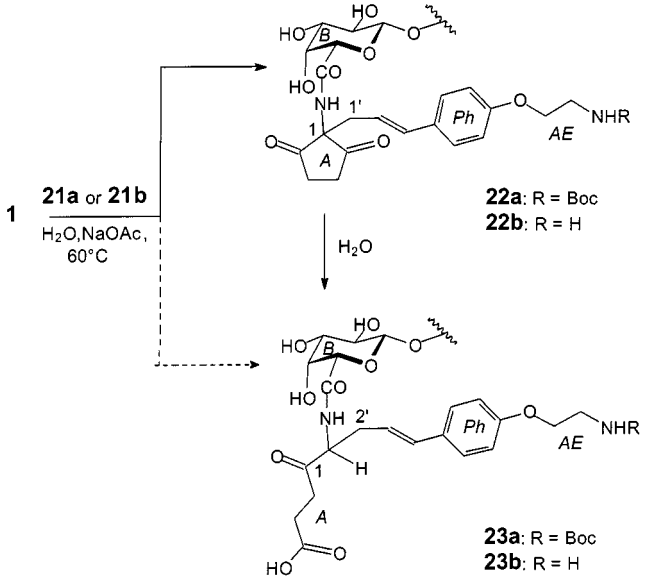
Scheme 6. a) refs.[10,17]; b) $SC(NH_2)_2$, EtOH, 70%; c) $SOCl_2$, THF; d) $SC(NH_2)_2$, acetonitrile; e) $SC(NH_2)_2$, acetonitrile, 88%

We therefore prepared the aminoethoxy-functionalized cinnamyl thiouronium bromide **21b** via **21a**, as summarized in Scheme 7. The only remarkable step in this sequence was the reduction of **20a** to give **20b**, which worked well under the conditions described by Sakaitani and Ohfune.[21]



Scheme 7. a) EtOH/H^+; b) $BrCH_2CH_2NHBoc$, K_2CO_3, DMF; c) $BF_3{\cdot}Et_2O$, DIBAL-H, CH_2Cl_2, −78 °C, 72%; d) PBr_3, CH_2Cl_2, −10 °C; e) $SC(NH_2)_2$, MeCN; f) HCl/EtOH, 20 °C, 82%

Treatment both of **21a** and of **21b** with moenomycin A (**1**) in buffered aqueous solution at pH = 5.7 gave the desired conjugates **22a** and **22b** in yields of 45 and 81%, respectively, based on **1** consumed (Scheme 8). The structures were confirmed by NMR and MS as described for **14b**.

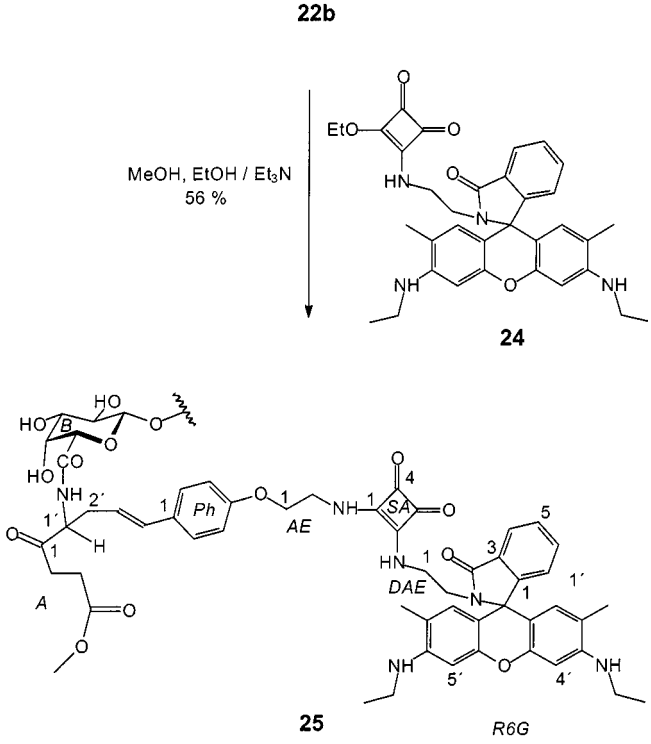


Scheme 8

In the course of the structure elucidation of **14b**, we made an interesting observation. After the NMR sample (in CD_3OD) had been stored in a refrigerator for 10 d, the ^1H NMR spectrum showed the presence of a mixture of two compounds, consisting of **14b** and a new product with signals at δ = 4.15 and 4.40 and somewhat different signals for the aromatic protons 2′-H and 3′-H. The CH_2-3^A and CH_2-4^A signals of **14b** (at δ = 2.78−2.88) were absent because of H/D exchange. We speculated that the cyclopentanedione ring in the second compound had been cleaved by a retro-Claisen-type reaction. This assumption was shown to be correct by performing the treatment of thiouronium salt **21b** with **1** at different pH values. At a pH of approximately 6.4, formation only of **22b** was observed by TLC (RP18, R_f = 0.26). After adjustment of the pH to 7.7 and further stirring at 60 °C for 25 h, however, a new product − **23b** (TLC, RP18, R_f = 0.37) − was obtained in 32% yield (based on **1** consumed). NMR and MS analysis of this product showed that the cyclopentanedione ring had been cleaved under the reaction conditions (retro-Claisen-type reaction) and a propoxycarbonyl group had been formed (Scheme 8). Similar results were obtained with **21a** (see Exp. Sect.). On cleavage of the cyclopentanedione ring, a new stereogenic center at C-1′ in **23a** and **23b** is formed (the change in the numbering of the carbon atoms in these compounds follows the IUPAC rules). Hence, compounds **23a** and **23b** exist as mixtures of two diastereomers that we were not able to separate by flash chromatography (FC). However, in the ^1H NMR spectra of **23a** and **23b** the well-separated signals of 3′-H and 4′-H of each diastereomer (1:1 ratio) and the signals of the CH_2-2′ protons (long-range coupling to C-3′ and C-4′) and of CH_2-2^A and CH_2-3^A (long-range coupling to C-1^A, *C*OOH^A) of each diastereoisomer could be assigned for each diastereoisomer with the aid of ^1H,^1H COSY and HMBC experiments. In the ^{13}C NMR spectra, instead of the two carbonyl carbon (C-2^A, C-5^A) signals of **22a** and **22b**, only one carbonyl carbon signal (C-1^A) was observed in each case for compounds **23a** and **23b**, together with the carboxylic group carbon signal ($COOH^A$). The 1′-H signal (broad signal in the region of δ = 4.50−4.69) could be identified because of the coupling of this proton with 2′-H (^1H,^1H COSY). The corresponding C-1′ signal was identified by its coupling with 1′-H (HMQC). The proton signals of the CH_2-2′ group were identified by ^1H,^1H COSY (coupling with 3′-H), while C-2′ in each diastereomer showed long-range couplings with 3′-H and 4′-H (HMBC). With the help of HMQC and HMBC experiments, practically all the carbon signals (except some overlapping sugar carbon and C-3^H, C-1^{AE}, and C-2^A signals) in **23a** and **23b** were assigned. Some corresponding carbon signals of each diastereomer had slightly different chemical shifts (see Exp. Sect.).

Thus, through control of the pH of the reaction mixture of thiouronium salt **21b** and moenomycin A (**1**), either **22b** − with the intact cyclopentanedione ring (pH = 5.74) − or **23b** − with the carboxypropionyl substituent (pH > 7.0) − could be obtained, in 81% and 37% yield, respectively (based on **1** consumed). The same behavior on treatment with **1** was observed with the Boc-protected aminothiouronium salt **21a**.

In order to demonstrate that the new amine **22b** could be used for the preparation of moenomycin conjugates, the coupling of **22b** to **24**[22] was studied (Scheme 9). The reaction was performed in methanol containing a small amount of ethanol (to make the solution homogeneous) in the presence of Et_3N at 20 °C. It was observed by TLC analysis that two products with very similar R_f values were formed, and these were isolated in 56% yield. As then shown by NMR analysis, a mixture of two diastereoisomers had been obtained. The formation of these diastereomers can be explained as described above. In the ^1H and ^{13}C NMR spectra of **25**, almost all hydrogen and carbon signals (with the exception of some broad and overlapping carbon and proton signals of the sugar part and units AE and DAE, $COOH^H$) were assigned with the aid of 2D NMR experiments, as described for **23a** and **23b**. The CH_3O^A groups (two diastereomers) gave rise to a broad unresolved ^1H NMR signal ($\delta = 3.67$), while two OCH_3 signals (51.48, $\delta = 51.42$, assigned by HMQC) appeared in the ^{13}C NMR spectrum.



Scheme 9

Antibiotic Properties of the New Moenomycin Derivatives in Comparison with 1 and 2b

All the new derivatives were antibiotically less active than moenomycin A itself, but still showed high activities, with MIC values in the range of 0.13 (**23b**) to 1.50 (**25**) μmol/L (Table 1). It therefore seemed justified to use them for the conjugation methods indicated above. The largest decrease in antibiotic activity (ca. twentyfold) was observed on conversion of the enolized cyclopentane-1,3-dione unit A of moenomycin A (**1**) into the derivatives alkylated in the 2-position (Table 1). The MIC value for the new amino-moenomycin derivative **22b** was almost the same as for **2b**. The Boc protection of the amino function in the moenomycin derivatives **22a** and **23a** had practically no influence on the antibiotic activity.

Table 1. Minimum inhibitory concentrations (MICs) of the moenomycin derivatives obtained from moenomycin A by the thiouronium salt method

Compound	MIC	
	μmol/L	μg/mL
1[10]	0.0069	0.029
2b[10]	0.15	0.27
14a	0.69	1.21
14b	0.28	0.49
23a	0.27	0.50
22a	0.58	1.08
23b	0.13	0.24
22b	0.15	0.28
25[a]	1.50	3.5

[a] Based on one measurement.

Conclusions

New amino derivatives of moenomycin A have been prepared by making use of thiouronium salt **21b**. Depending on the pH value of the reaction mixture, the conjugates had structures of type **22b** or of type **23b**. In one example it was demonstrated that a fluorescent reporter group could be introduced selectively at the amino group through a squaric acid linker. An appreciable degree of antibiotic activity was retained in the conjugates.

Experimental Section

General: All air- 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 aluminium 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 with Na_2SO_4, and removal of solvent by distillation in a rotary evaporator (bath temperature 35 °C). Solvents were purified by standard techniques. The following materials and methods were used for chromatographic separations: flash chromatography (FC):[23] 32−63 μm silica gel (ICN Biomedicals) and LiChroprep 40−63 μm RP-18 material (Merck), Kieselguhr (Merck); analytical TLC: Merck precoated 60 F_{254} silica gel and RP-18 F_{254s} plates (0.2 mm), spots were identified under a UV lamp ($\lambda = 254$ nm, Camag 29 200), with a 2.22 mol/L H_2SO_4 solution containing $Ce(SO_4)_2 \cdot 4H_2O$ (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 with an anisaldehyde reagent for carbohydrates [2 mL of anisaldehyde, 8 mL of conc. H_2SO_4 in ethanol (190 mL)].Analytical HPLC was performed with a HPLC system (Jasco) consisting of an intelligent PU-980 HPLC pump, a DG-980−053 line degasser, an LG-980−02 ternary gradient unit, and an MD-910 multiwavelength detector. 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 250 × 4.6 mm, Nucleosil 300, C18, 5 µm column (Jasco) with a pre-column was used. Ultrafiltration (UF) was performed in gas-pressurized (N_2, 3.5 bar) stirred ultrafiltration cells (Amicon, model 8050, 50-mL cell capacity and model 8400, 400-mL capacity) with YM3 membrane type (Amicon, molecular weight cut-off 3000 Da). NMR and MS equipment: NMR: DRX 400 (Bruker), DRX 600 (Bruker), GEMINI 200 (Varian), GEMINI 2000 (Varian); for the description of the NMR spectra, the protons and carbon atoms are indexed according to the indices in the formulae. Mass spectrometry: FAB MS: VG Autospec (Fisons, matrix: 3-nitrobenzyl alcohol), ESI MS: FT ICR MS ApeX II [Bruker Daltonics, solvent: water/acetonitrile (1:1) or water/methanol (1:1)], MALDI TOF MS: VOYAGER™ spectrometer [PerSeptive Biosystems, matrix: 2,4,6-trihydroxyacetophenone from water/acetonitrile (1:1) solution]. Two masses are always communicated after the molecular formula; the first is that calculated with the International Atomic Masses, the second the mono-isotopic mass. IR: Genesis FTIR (ATI Mattson). UV: DU-650 (Beckman). Fluorescence spectra were recorded with a Fluoromax-2 (SPEX). The fluorescence excitation and emission were corrected according to the lamp spectrum and the photomultiplier sensitivity, respectively. The maxima of the excitation spectra indicate the UV/Vis absorption maxima. The MIC (minimum inhibitory concentration) values against seven different *Staphylococcus aureus* strains (ATCC 25923, ATCC 29213, MRSA 1309, SG 511, petroleum etherG 18, petroleum etherG 5, KNS petroleum etherG 5) were determined by a serial double microdilution 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.

2-[(*E*)-3-(4-Methoxyphenyl)-2-butenyl]thiouronium Acetate (7): Compound **7** was prepared as described in refs.[16,19]. M.p. 141−142 °C (ref.[16] 145−147 °C). IR (KBr): $\tilde{\nu}$ = 3550, 1633, 1604, 1569, 1511, 1409, 1249, 1178, 829 cm^{-1}. UV (methanol): λ_{max} (ε) = 240 (39312), 308 nm (6960). ^{1}H NMR (200 MHz, CD_3OD): δ = 1.91 (s, 3 H, CH_3COO$^-$), 2.14 (s, long range coupling, $J_{4'\text{-}1'}$ = 0.4 Hz, $J_{4'\text{-}2'}$ = 1.3 Hz, 3 H, CH_3-4′), 3.80 (s, 3 H, CH_3O), 4.04 (d, $J_{1'\text{-}2'}$ = 7.7 Hz, 2 H, CH_2-1′), 5.81 (tq, $J_{2'\text{-}1'}$ = 7.7 Hz, $J_{2'\text{-}4'}$ = 1.3 Hz, 1 H, 2′-H), 6.89 ($J_{3\text{-}2}$ = 9.2 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.37 ($J_{2\text{-}3}$ = 9.2 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (50 MHz, CD_3OD): δ = 16.4 (C-4′), 31.3 (C-1′), 56.0 (*C*H_3O), 115.1 (C-3^{Ph}, C-5^{Ph}), 117.5 (C-2′), 128.3 (C-2^{Ph}, C-6^{Ph}), 136.01 (C-1^{Ph}), 143.7 (C-3′), 161.3 (C-4^{Ph}). $C_{12}H_{16}N_2OS$ (base, 236.33, 236.09), FAB MS: *m*/*z* = 237.1 [M + H]$^+$, 161.1 [M − $SC(NH_2)_2$ + H]$^+$.

2-[(*E*)-3-(4-Methoxyphenyl)but-2-enyl]-2-methylcyclopentane-1,3-dione (8):[16,19] R_f = 0.93 (toluene/ethyl acetate/methanol, 10:5:2). ^{1}H NMR (200 MHz, CD_3OD): δ = 1.13 (s, 3 H, 2-CH_3), 1.96 (d, $J_{4'\text{-}2'}$ = 1.2 Hz, 3 H, CH_3-4′), 2.50 (d, $J_{1'\text{-}2'}$ = 8.0 Hz, 2 H, CH_2-1′), 2.74 (m, 4 H, CH_2-4, CH_2-5), 3.78 (s, 3 H, CH_3O), 5.49 (tq, $J_{2'\text{-}1'}$ = 8.0 Hz, $J_{2'\text{-}4'}$ = 1.2 Hz, 1 H, 2′-H), 6.85 ($J_{3\text{-}2}$ = 8.8 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.26 ($J_{2\text{-}3}$ = 8.8 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (50 MHz, CD_3OD): δ = 16.2 (2-*C*H_3), 18.4 (C-4′), 36.2 (C-4, C-5), 36.3 (C-1′), 55.7 (*C*H_3O), 58.1 (C-2), 114.6 (C-3^{Ph}, C-5^{Ph}), 120.2 (C-2′), 127.8 (C-2^{Ph}, C-6^{Ph}), 137.1 (C-1^{Ph}), 139.8 (C-3′), 160.4 (C-4^{Ph}), 218.7 (C-1, C-3).

2-Acetamido-2-[(*E*)-3-(4-Methoxyphenyl)but-2-enyl]cyclopentane-1,3-dione (10): 2-Acetaminocyclopentane-1,3-dione (**9**)[14] (15.5 mg, 0.1 mmol) and thiouronium salt **7** (29.6 mg, 0.1 mmol) in water (0.5 mL) and toluene (0.3 mL) were stirred at 60 °C for 6 h. The solvents were removed under reduced pressure and the residue was purified by FC (toluene/ethyl acetate/methanol, 10:5:2). The crude product was further purified by FC (toluene/ethyl acetate, 2:1, R_f = 0.19) to give 22 mg (71%) of pure **10** as an oil. R_f = 0.58 (toluene/ethyl acetate/methanol, 10:5:2). IR (KBr): $\tilde{\nu}$ = 3432, 3274, 1729, 1629, 1511, 1288, 1245 cm^{-1}. UV (methanol): λ_{max} (ε) = 256 nm (15294). ^{1}H NMR (200 MHz, CD_3OD): δ = 1.93 (d, $J_{4'\text{-}2'}$ = 0.9 Hz, 3 H, CH_3-4′), 1.95 (s, 3 H, CH_3CONH), 2.62 (d, $J_{1'\text{-}2'}$ = 7.7 Hz, 2 H, CH_2-1′), 2.75 (s, 4 H, CH_2-4, CH_2-5), 3.77 (s, 3 H, CH_3O), 5.59 (t, $J_{2'\text{-}1'}$ = 7.7 Hz, 1 H, 2′-H), 6.86 (d, $J_{3\text{-}2}$ = 7.9 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.29 (d, $J_{2\text{-}3}$ = 7.9 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (50 MHz, CD_3OD): δ = 16.2 (C-4′), 20.5 (*C*H_3CONH), 32.5 (C-1′), 35.7 (C-4, C-5), 55.7 (*C*H_3O), 67.1 (C-2), 114.7 (C-3^{Ph}, C-5^{Ph}), 116.8 (C-2′), 127.8 (C-2^{Ph}, C-6^{Ph}), 136.5 (C-1^{Ph}), 141.5 (C-3′), 160.7 (C-4^{Ph}), 172.6 (CH_3*C*ONH), 214.0 (C-1, C-3). $C_{18}H_{21}NO_4$ (315.36, 315.15), FAB MS: *m*/*z* = 316.1 [M + H]$^+$.

(*R*)-3-[(*N*-{1-[(*E*)-3-(4-Methoxyphenyl)-2-butenyl]-2,5-dioxocyclopentyl}-β-D-galactopyranuronamidosyl-(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 (14a): Moenomycin A (**1**) (99 mg, 0.06 mmol) and **7** (28 mg, 0.09 mmol) were stirred under argon in water (1.5 mL) at 60 °C. Progress of the reaction was monitored by TLC (1-propanol/H_2O, 7:2). After 8 h, the TLC spot of **14a** appeared. Even after 36 h, however, the reaction was not complete. More thiouronium salt **7** (18 mg, total 46 mg, 0.15 mmol) and water (1 mL, total 2.5 mL) were added, and heating and stirring were continued for 49 h. Even then, moenomycin A was not completely consumed. Heating was stopped and the reaction mixture was allowed to cool to ambient temperature. The excess of thiouronium salt and thiourea formed in the reaction were then removed by ultrafiltration (4 × 50 mL). After freeze-drying, 108 mg of a solid product was obtained, and this was purified twice by FC (1-propanol/H_2O, 5:1) to give 57 mg (55%) of pure **14a**, with 17 mg of **1** recovered. R_f = 0.57 (1-propanol/H_2O, 7:2). UV: λ_{max} = 257 (ε = 10815, methanol), 256 (methanol + HCl), 253 (methanol + NaOH), for **1**: λ_{max} 258 nm (ε = 17305, methanol), 242 (methanol + HCl), 258 (methanol + NaOH).[25] ^{1}H NMR (600 MHz, CD_3OD, HMQC): δ = 0.99 (s, 6 H, CH_3-23^I, CH_3-24^I), 1.26 (s, 3 H, $CH_3{}^F$), 1.39 (m, 2 H, CH_2-9^I), 1.42 (d, 3 H, CH_3-6^C, *J* = 4.7 Hz), 1.61 (s, 3 H, CH_3-20^I), 1.62 (s, 3 H, CH_3-21^I), 1.68 (s, 3 H, CH_3-19^I), 1.78 (s, 3 H, CH_3-25^I), 1.92 (m, 2 H, CH_2-10^I), 1.96 (s, 6 H, CH_3-4′, CH_3CONH^E or CH_3CONH^C), 2.00−2.20 (m, 11 H, CH_2-4^I, CH_2-5^I, CH_2-15^I, CH_2-16^I and 2.03 (s, CH_3CONH^C or CH_3CONH^E), 2.63−2.86 (m, 8 H, CH_2-1′, CH_2-3^A, CH_2-4^A and 2.70 (d, $J_{12\text{-}13}$ = 7.3 Hz, CH_2-12^I)], 3.20−4.60 [broad signals of the sugar protons with 3.80 (s, 3 H, OCH_3), 4.09 (5^B-H, 4^B-H), 4.46 (d, *J* = 6.8 Hz, 1^D-H)], 4.68 (d, *J* = 6.8 Hz, 2 H, CH-22^I), 5.11 (bt, $J_{17\text{-}16}$ = 6.3 Hz, 2 H, 17^I-H, and 3^F-H), 5.15 (t, $J_{13\text{-}12}$ = 7.3 Hz, 1 H, 13^I-H), 5.33 (broad signal, 1 H, 6^I-H), 5.39 (d, $J_{7\text{-}6}$ = 15.7 Hz, 1 H, 7^I-H), 5.44 (broad signal, 1 H, 2^I-H), 5.65 (t, $J_{2'\text{-}1'}$ = 7.9 Hz, 1 H, 2′-H), 5.91 (broad signal, 1 H, 1^F-H), 6.88 (d, $J_{3\text{-}2}$ = 8.4 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.35 (d, $J_{2\text{-}3}$ = 8.4 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (150 MHz, CD_3OD, HMQC): δ = 16.2 (C-21^I), 16.3 (C-4′), 16.5 ($CH_3{}^F$), 17.8 (C-6^C, C-20^I), 23.4 (*C*H_3CONH^C and *C*H_3CONH^E), 24.2 (C-25^I), 25.9 (C-19^I), 27.7 (C-16^I), 27.9 (C-23^I, C-24^I), 32.4 (C-10^I), 32.8 (C-5^I), 33.2 (C-1′), 33.5 (C-4^I), 35.8, 35.9 (C-3^A, C-4^A), 35.9 (C-12^I), 36.5 (C-8^I), 40.9 (C-15^I), 42.9 (C-9^I), 55.8 (O*C*H_3), 56.2 (C-2^E), 56.7 (C-2^C), 62.9 (C-6^D), 67.1 (C-1^I or C-1^A), 69.9 (broad, C-3 ^{H}), 70.5 (C-4^B), [72.0, 72.3, 72.6, 73.6, 73.7,

74.1, 74.2, 74.3, 75.1, 75.3 (C-5^{B}), 76.3 (broad), 78.0, 78.8 (broad), 82.5 (broad), (unassigned signals of the sugar carbon atoms)], 84.7 (C-4^{C}), 95.9 (C-1^{F}), 103.4, 103.9, 104.4, 104.6 (C-1^{C}, C-1^{E}, C-1^{D}, C-1^{B}), 109.3 (C-22^{I}), 114.7 (C-3^{Ph}, C-5^{Ph}), 116.9 (C-2′), 123.5 (C-13^{I}), 125.4 (C-17^{I}), 126.9 (C-6^{I}), 127.9 (C-2^{Ph}, C-6^{Ph}), 132.2 (C-18^{I}), 136.6 (C-3′), 137.3 (C-14^{I}), 141.5 (C-3^{I}), 141.6 (C-7^{I}), 151.1 (C-11^{I}), 159.2 (O*C*ONH$_2$^{F}), 160.6 (C-4^{Ph}), 170.5 (*C*ONH^{B}), 173.7 (*C*ONH$_2$^{F}), 174.1, 174.3 (CH$_3$*C*ONH^{C} and *C*H$_3$CONH^{E}), 212.9, 213.8 (C-2^{A}, C-5^{A}). $C_{80}H_{120}N_5O_{35}P$ (1742.81, 1741.75), ESI ICR MS: *m*/*z* = 1740.74642 (calcd. 1740.74225) [M − H]$^-$, 869.86951 (calcd. 869.86780) [M − 2 H]$^{2-}$.

2-[(*E*)-Cinnamyl]thiouronium Bromide (18b):[26,19] M.p. 195−197 °C (ref.[26] 196−197 °C). IR (KBr): $\tilde{\nu}$ = 3320−3370, 3120−3270, 1636, 1440, 986, 747, 688, 622, 469 cm^{-1}. UV (methanol): λ_{max} (ε) = 254 nm (24756). ^{1}H NMR (200 MHz, [D$_6$]DMSO/D$_2$O, 10:1): δ = 3.98 (d, $J_{1'\text{-}2'}$ = 7.0, Hz 2 H, CH$_2$-1′), 6.25 (dt, $J_{2'\text{-}1'}$ = 7.0 Hz, $J_{2'\text{-}3'}$ = 15.7 Hz, 1 H, 2′-H), 6.69 (d, $J_{3'\text{-}2'}$ = 15.7 Hz, 1 H, 3′-H), 7.23−7.44 (m, 5 H, 2-H, 3-H, 4-H, 5-H, 6-H). ^{13}C NMR (50 MHz, [D$_6$]DMSO/D$_2$O, 10:1): δ = 33.7 (C-1′), 123.1 (C-2′), 127.4 (C-2, C-6), 129.3 (C-4), 129.8 (C-3, C-5), 135.2, 136.6 (C-1, C-3′), 170.1 [S*C*(NH)NH$_2$). $C_{10}H_{12}N_2S$ (base, 192.27, 192.07), FAB MS: *m*/*z* = 193.0 [M + H]$^+$, 117.1 [M − SC(NH$_2$)$_2$ + H]$^+$.

2-(Benzyl)thiouronium Chloride (17b):[27,19] M.p. 145 °C (aq. HCl) (ref.[27] 148 °C). IR (KBr): $\tilde{\nu}$ = 3400 (broad), 3070−3200, 1640, 1420, 760, 703, 677, 568 cm^{-1}. UV (methanol): λ_{max} (ε) = 209 nm (14864). ^{1}H NMR (200 MHz, D$_2$O): δ = 4.35 (s, 2 H, -CH$_2$−S-), 7.33−7.44 (m, 5 H, 2-H, 3-H, 4-H, 5-H, 6-H). ^{13}C NMR (50 MHz, D$_2$O): δ = 35.5 (−*C*H$_2$−S), 128.9 (C-4), 129.4, 129.6 (C-3 and C-5, C-2 and C-6), 134.5 (C-1), 171.0 [S*C*(NH)NH$_2$). $C_8H_{10}N_2S$ (base, 166.24, 166.06), FAB MS: *m*/*z* = 167.0 [M + H]$^+$, 91.1 [C$_6$H$_5$CH$_2$]$^+$.

2-[(*E*)-3-Phenyl-2-butenyl]thiouronium Acetate (16): Compound **16** was prepared from acetophenone (**15**) as described for **7**. M.p. 163−164 °C (water). IR (KBr): $\tilde{\nu}$ = 3385−3275, 3177−3100, 1613, 1470, 1414, 1084, 729 cm^{-1}. UV (methanol): λ_{max} (ε) = 241 nm (29303). ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 1.79 (s, 3 H, C*H*$_3$COO$^-$), 2.05 (s, 3 H, CH$_3$-4′), 3.77 (d, $J_{1'\text{-}2'}$ = 8.0 Hz, 2 H, CH$_2$-1′), 5.85 (t, $J_{2'\text{-}1'}$ = 8.0 Hz, 1 H, 2′-H), 7.05 (broad s, NH, NH$_2$), 7.20−7.40 (m, 5 H, 2^{Ph}-H, 3^{Ph}-H, 4^{Ph}-H, 5^{Ph}-H, 6^{Ph}-H). ^{1}H NMR (200 MHz, CD$_3$OD): δ = 1.91 (s, 3 H, C*H*$_3$COO$^-$), 2.17 (long range coupling, $J_{4'\text{-}2'}$ = 1.4 Hz, $J_{4'\text{-}1'}$ = 0.7 Hz, 3 H, CH$_3$-4′), 4.06 (d, $J_{1'\text{-}2'}$ = 7.8 Hz, 2 H, CH$_2$-1′), 5.87 (td, $J_{2'\text{-}1'}$ = 7.8 Hz, $J_{1'\text{-}4'}$ = 0.7 Hz, 1 H, 2′-H), 7.13 (broad s, NH, NH$_2$), 7.29−7.45 (m, 5 H, 2^{Ph}-H, 3^{Ph}-H, 4^{Ph}-H, 5^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (50 MHz, CD$_3$OD, ^{1} H,^{13}C COSY): δ = 16.3 (C-4′), 24.1 (*C*H$_3$COO$^-$), 30.9 (C-1′), 119.1 (C-2′), 126.8 (C-3^{Ph}, C-5^{Ph}), 128.8 (C-1^{Ph}), 129.4 (C-2^{Ph}, C-6^{Ph}), 143.3, 143.9 (C-3′, C-4^{Ph}), 172.4 [S*C*(NH)NH$_2$), 185.2 (CH$_3$*C*OO$^-$). $C_{11}H_{14}N_2S$ (base, 206.30, 206.09), FAB MS: *m*/*z* = 230.0 [M + Na]$^+$, 207.1 [M + H]$^+$.

Treatment of Moenomycin A with 16 and 17b: Mixtures of moenomycin A (30 mg, 0.019 mmol) and thiouronium salts **16** and **17b** (each 2.5 equiv.) in water (5 mL) were stirred under argon at 60 °C . TLC monitoring (1-propanol/H$_2$O, 7:2) indicated no product formation even after 24 h.

2-[(*E*)-4-Methoxycinnamyl]thiouronium Bromide (13): A solution of PBr$_3$ (0.218 g, 0.075 mL, 0.8 mmol) in dry CH$_2$Cl$_2$ (1 mL) was slowly added under an argon and at −10 °C to a stirred solution of 4-methoxycinnamyl alcohol[18] (0.36 g, 2.2 mmol) in dry CH$_2$Cl$_2$ (4 mL). The reaction mixture was then stirred for an additional 20 min and quenched with a saturated aqueous solution of NaHCO$_3$. The product was separated from the aqueous layer by extraction with diethyl ether. The ethereal solution was washed with water and dried with sodium sulfate. After filtration and solvent removal, 0.45 g of a white solid was obtained. The solid was dissolved in acetonitrile (2 mL), and a solution of thiourea (0.1 g, 1.3 mmol) in acetonitrile (2 mL) was added. After 1−2 min, a solid precipitated from the clear solution. The precipitate was collected by filtration, washed with acetonitrile and dried in vacuum to provide 0.338 g (85% based on thiourea) of pure **12**. M.p. 148 °C (decomp., acetonitrile). IR (KBr): $\tilde{\nu}$ = 3600−3200 (broad, NH, NH$_2$), 3200−3000 (C-H^{Ar}), 1637, 1511, 1438, 1250, 1175, 609 cm^{-1}. UV (methanol): λ_{max} (ε) = 230 (20424), 274 (2763), 283 (s) nm (2218). ^{1}H NMR (200 MHz, CD$_3$OD): δ = 3.78 (s, 3 H, OC*H*$_3$), 4.00 (dd, $J_{1'\text{-}2'}$ = 7.3 Hz, $J_{1'\text{-}3'}$ = 1.1 Hz, 2 H, 1′-H) 6.12 (dt, $J_{2'\text{-}3'}$ = 15.8 Hz, $J_{2'\text{-}1'}$ = 7.3 Hz, 1 H, 2′-H), 6.70 (d, $J_{3'\text{-}2'}$ = 15.8 Hz, 1 H, 3′-H), 6.89 ($J_{3\text{-}2}$ = 8.8 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.35 ($J_{2\text{-}3}$ = 8.8 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (50 Hz, CD$_3$OD, APT): δ = 35.0 (C-1′), 55.8 (O*C*H$_3$), 115.2 (C-3^{Ph}, C-5^{Ph}), 119.7 (C-2′), 129.1 (C-2^{Ph}, C-6^{Ph}), 130.0 (C-1^{Ph}), 136.5 (C-3′), 161.5 (C-4^{Ph}), 172.4 [S*C*(NH)NH$_2$). $C_{11}H_{14}N_2OS$ (base, 222.30, 222.08), FAB MS: *m*/*z* = 223.1 [M + H]$^+$, 147.1 [M − SC(NH$_2$)$_2$ +H]$^+$.

(*R*)-3-[(*N*-{1-[(*E*)-3-(4-Methoxyphenyl)propenyl]-2,5-dioxocyclopentyl}-β-D-galactopyranuronamidosyl-(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 (14b): A solution of moenomycin A (1, 50 mg, 0.032 mmol), sodium acetate (9 mg), and thiouronium bromide (13, 14.3 mg, 0.048 mmol) in water (1.5 mL) was stirred under argon at 60 °C for 30 h. Progress of the reaction was monitored by TLC (1-propanol/H$_2$O, 7:2) and HPLC (R_t = 29.15 min, $R_t^{\text{moenomycin A}}$ = 13.49 min, flow 0.5 mL/min). Since the reaction was not complete, more 13 (9.6 mg, 0.032 mmol), sodium acetate (7 mg), and water (0.5 mL) were added and the reaction mixture was stirred at 60 °C for an additional 30 h (together 60 h). Water was then removed by lyophilization, and the crude product was purified twice by FC (1-propanol/H$_2$O, 6:1). Solvent evaporation under reduced pressure and water lyophilization gave 31.5 mg (57%) of pure (HPLC) **14b**. R_f = 0.50 (1-propanol/H$_2$O, 7:2). UV (methanol): λ_{max} (ε) = 264 nm (27970). ^{1}H NMR (600 MHz, CD$_3$OD, ^{1}H,^{1}H COSY, HMQC, HMBC): δ = 0.98 (s, 6 H, CH$_3$-23^{I}, CH$_3$-24^{I}), 1.27 (s, 3 H, CH$_3$^{F}), 1.38 (m, 2 H, CH$_2$-9^{I}), 1.42 (d, *J* = 5.8 Hz, 3 H, CH$_3$-6^{C}), 1.61 (s, 3 H, CH$_3$-20^{I}), 1.62 (s, 3 H, CH$_3$-21^{I}), 1.68 (s, 3 H, CH$_3$-19^{I}), 1.77 (s, 3 H, CH$_3$-25^{I}), 1.91 (s), 1.91 (m, 2 H, CH$_2$-10^{I}), 1.94 (s, 3 H, C*H*$_3$CONH^{C} or C*H*$_3$CONH^{E}), 2.00−2.20 (m, 11 H, CH$_2$-4^{I}, CH$_2$-5^{I}, CH$_2$-15^{I}, 16^{I} and 2.07 (s, C*H*$_3$CONH^{E} or C*H*$_3$CONH^{C}), 2.60−2.69 (m, 2 H, CH$_2$-1′), 2.70 (d, $J_{12\text{-}13}$ = 7.3 Hz, 2 H, CH$_2$-12^{I}), 2.78−2.88 (m, 2 H, CH$_2$-3^{A}, CH$_2$-4^{A}), 3.40−4.65 (broad signals of the sugar protons with 3.81 (s, 3 H, OCH$_3$), 4.68 (d, *J* = 8.9 Hz, CH$_2$-22^{I}), 5.11 (broad t, $J_{17\text{-}16}$ = 5.5 Hz, 2 H, 17^{I}-H and 5.12 (3^{F}-H), 5.14 (t, $J_{13\text{-}12}$ = 7.3 Hz, 1 H, 13^{I}-H), 5.31 (dt, $J_{6\text{-}7}$ = 15.2 Hz, $J_{6\text{-}5}$ = 6.7 Hz, 1 H, 6^{I}-H), 5.39 (d, $J_{7\text{-}6}$ = 15.2 Hz, 1 H, 7^{I}-H), 5.45 (broad signal, 1 H, 2^{I}-H), 5.89 (broad signal, 1 H, 1^{F}-H), 6.04 (dt, $J_{2'\text{-}3'}$ = 15.7 Hz, $J_{2'\text{-}1'}$ = 7.6 Hz, 1 H, 2′-H), 6.46 (d, $J_{3'\text{-}2'}$ = 15.7 Hz, 1 H, 3′-H), 6.89 (d, $J_{3\text{-}2}$ = 8.9 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.35 (d, $J_{2\text{-}3}$ = 8.9 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (150 MHz, CD$_3$OD, APT, HMQC, HMBC): δ = 16.2 (C-21^{I}), 16.4 (CH$_3$^{F}), 17.8 (C-6^{C}), 17.9 (C-20^{I}), 23.5 (*C*H$_3$CONH^{C}, *C*H$_3$CONH^{E}), 24.1 (C-25^{I}), 26.0 (C-19^{I}), 27.7 (C-16^{I}), 27.9 (C-23^{I}, C-24^{I}), 32.3 (C-10^{I}), 32.7 (C-5^{I}), 33.5 (C-4^{I}), 34.8 (broad, C-3^{A}, C-4^{A}), 35.9 (C-12^{I}), 36.5 (C-8^{I}), 36.9 (C-1′), 40.8 (C-15^{I}), 42.9 (C-9^{I}), 55.9 (O*C*H$_3$), 56.1, 56.7 (C-2^{E}, C-2^{C}), 62.0*, 62.7 (O*C*H$_2$^{D}), 66.9* (C-1^{I}), 68.2 (C-1^{A}), 70.4, 71.3*, 71.6, 72.3, 72.5 (C-

5^{C}), 73.5*, 73.8, 73.9, 74.1, 74.2*, 74.3 (C-5^{F}), 75.0, 75.3, 76.1 (C-3^{F}), 77.8, 78.0, 78.6, 80.9 (broad, C-2 ^{H}), 82.2, 84.7 (C-4^{C}, C-4^{E}), 95.8 (C-1^{F}), 103.3 (C-1^{C}), 103.9 (C-1^{E}), 104.3, 104.5 (C-1^{D}, C-1^{B}), 109.3 (C-22^{I}), 115.0 (C-3^{Ph}, C-5^{Ph}), 118.9 (C-2′), 123.1 (broad, C-2^{I}), 123.5 (C-17^{I}), 125.3 (C-13^{I}), 126.9 (C-6^{I}), 128.6, 128.8 (C-2^{Ph}, C-6^{Ph}), 130.8 (C-1^{Ph}), 132.3 (C-18^{I}), 136.5 (C-3′), 137.4 (C-14^{I}), 141.6 (C-7^{I}), 151.1 (C-11^{I}), 159.2 (O*C*ONH$_2$^{F}), 160.9 (C-4^{Ph}), 170.5 (*C*ONH^{B}), 173.9, 173.9 (CH$_3$*C*ONH^{C} or CH$_3$*C*ONH^{E}, *C*ONH$_2$^{F}), 174.4 (CH$_3$*C*ONH^{E} or CH$_3$*C*ONH^{C}), 177.8 (*C*O$_2$H^{H}), 180.6, 212.8, 213.7 (C-2^{A}, C-5^{A}). * Signals of secondary or quaternary carbon atoms (APT) in the δ = 60−80 range. $C_{79}H_{118}N_5O_{35}P$ (1728.79, 1727.73), ESI ICR MS: *m*/*z* = 1748.72412 (calcd. 1748.70870) [M + Na − 2H]$^-$, 1726.74254 (calcd. 1726.72660) [M − H]$^-$, 862.85829 (calcd. 862.85935) [M − 2H]$^{2-}$. FAB MS: *m*/*z* = 1788.5 [M + Na + K − H]$^+$, 1772.5 [M + 2Na − H]$^+$, 1766.5 [M + K]$^+$, 1750.5 [M + Na]$^+$. MALDI TOF MS: *m*/*z* = 1748.3 [M − 2 H + Na]$^-$, 1726.4 [M − H]$^-$.

2-(4-Methoxybenzyl)thiouronium Chloride (17d): The crude benzyl chloride **17c**[28] was converted into **17d** as described for **13**. Drying in vacuum gave 0.674 g (67% based on thiourea) of pure **17d**. M.p. 165−166 °C (acetonitrile) (ref.[29] 161−163 °C). IR (KBr): $\tilde{\nu}$ = 3247, 3077, 2967, 1646, 1511, 1430, 1303, 1245, 1180, 1025, 840, 728, 703 cm^{-1}. UV (methanol): λ_{max} (ε) = 230 (18471). ^{1}H NMR (200 MHz, CD$_3$OD): δ = 3.78 (s, 3 H, OC*H*$_3$), 4.40 (s, 2 H, −C*H*$_2$−S−), 6.91 (*J*$_{3\text{-}2}$ = 8.8 Hz, 2 H, 3-H, 5-H), 7.34 (*J*$_{2\text{-}3}$ = 8.8 Hz, 2 H, 2-H, 6-H). ^{13}C NMR (50 MHz, CD$_3$OD): δ = 36.0 (−*C*H$_2$−S−), 55.8 (O*C*H$_3$), 115.5 (C-3, C-5), 126.7 (C-1), 131.6 (C-2, C-6), 161.4 (C-4), 172.4 [S*C*(NH)NH$_2$]. $C_9H_{12}N_2OS$ (base, 196.26, 196.07), FAB MS: *m*/*z* = 197.1 [M + H]$^+$, 121.1 [M − SC(NH$_2$)$_2$ + H]$^+$.

(*R*)-3-({*N*-[1-(4-Methoxybenzyl)-2,5-dioxocyclopentyl]-β-D-galactopyranuronamidosyl-(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 (Formula Not Shown): A solution of moenomycin A (**1**, 50 mg, 0.032 mmol), sodium acetate (8 mg), and thiouronium salt **17d** (12.8 mg, 0.054 mmol) in water (1.5 mL) was stirred under argon at 60 °C for 22 h. The reaction was monitored by TLC (1-propanol/H$_2$O, 7:2). No product formation was observed. The temperature was then increased to 75 °C and the mixture was stirred at this temperature for an additional 52 h. TLC (1-propanol/H$_2$O, 7:2) indicated product formation only to a minor extent. A further portion of **17d** (15 mg 0.064 mmol, total 0.118 mmol, 3.7equiv.), sodium acetate (12 mg), and water (0.5 mL) were added, and the reaction mixture was stirred at 75 °C for another 70 h. According to TLC, moenomycin A was still present. The solvent was removed by lyophilization and the residue was purified by FC (1-propanol/H$_2$O, 7:2) to provide 4 mg (7.3%) of the pure conjugate (formula not shown). R_f = 0.51 (1-propanol/H$_2$O, 7:2). Only characteristic signals in ^{1}H NMR and ^{13}C NMR could be assigned, due to the lack of sufficient amounts of the compound. ^{1}H NMR (600 MHz, CD$_3$OD, ^{1} H,^{1}H COSY): δ = 0.99 (s, 6 H, CH$_3$-23^{I}, CH$_3$-24^{I}), 1.27 (s, 3 H, CH$_3$^{F}), 1.39 (m, CH$_2$-9^{I}), 1.44 (d, *J* = 5.7 Hz, 3 H, CH$_3$-6^{C}), 1.62 (s, 3 H, CH$_3$-20^{I}), 1.63 (s, 3 H, CH$_3$-21^{I}), 1.68 (s, 3 H, CH$_3$-19^{I}), 1.77 (s, 3 H, CH$_3$-25^{I}), 1.92 (m, CH$_2$-10^{I}), 1.97 (broad s, 3 H, C*H*$_3$CONH^{C} or C*H*$_3$CONH^{E}), 2.00−2.15 (m, 11 H, CH$_2$-4^{I}, CH$_2$-5^{I}, CH$_2$-15^{I}, CH$_2$-16^{I}, and 2.07 (broad s, C*H*$_3$CONH^{E} or C*H*$_3$CONH^{C}), 2.71 (d, *J*$_{12\text{-}13}$ = 7.3 Hz, 2 H, CH$_2$-12^{I}), 3.04 (s, 2 H, C*H*$_2$^{Bz}), 3.25−4.60 [broad signals of the sugar protons with 3.78 (s, OCH$_3$)], 4.68 (d, *J* = 7.8 Hz, 2 H, CH$_2$-22^{I}), 5.11 (t, *J*$_{17\text{-}16}$ = 7.1 Hz, 1 H, 17^{I}-H), 5.16 (t, *J*$_{13\text{-}12}$ = 7.3 Hz, 1 H, 13^{I}-H), 5.33 (m, 1 H, 6^{I}-H), 5.39 (d, *J*$_{7\text{-}6}$ = 15.1 Hz, 7^{I}-H), 5.45 (broad signal, 1 H, 2^{I}-H), 5.89 (broad signal, 1 H, 1^{F}-H), 6.88 (d, *J*$_{3\text{-}2}$ = 8.9 Hz, 2 H, 3^{Bz}-H, 5^{Bz}-H), 7.02 (d, *J*$_{2\text{-}3}$ = 8.9 Hz, 2 H, 2^{Bz}-H, 6^{Bz}-H). ^{13}C NMR (600 MHz, CD$_3$OD, APT, HMQC): δ = 16.2 (C-21^{I}), 16.5 (CH$_3$^{F}), 17.8 (C-6^{C})*, 17.8 (C-20^{I}), 23.4, 23.4 (*C*H$_3$CONH^{C,} *C*H$_3$CONH^{E})*, 24.1 (C-25^{I})*, 25.9 (C-19^{I}), 27.7 (C-16^{I}), 27.9 (C-23^{I}, C-24^{I}), 32.4 (C-10^{I}), 32.8, 33.6 (C-4^{I}, C-5^{I}), 33.0 (C-3^{A}, C-4^{A}), 35.9 (C-12^{I}), 36.5 (C-8^{I}), 40.1 (CH$_2$^{Bz})*, 40.9 (C-15^{I}), 42.9 (C-9^{I}), 55.7 (OCH$_3$), 62.1 (C-6^{D}), 67.6 (C-1^{I} or C-3 ^{H}), 70.0−80.0 (broad signals of the sugar carbon atoms), 109.3 (C-22^{I}), 115.3 (C-3^{Bz}, C-5^{Bz}), 123.5 (C-13^{I}), 125.4 (C-17^{I}), 126.9 (C-6^{I}), 132.2 (C-18^{I}), 132.4 (C-2^{Bz}, C-6^{Bz}), 137.4 (C-14^{I}), 141.6 (C-7^{I}), 151.1 (C-11^{I}), 160.9 (O*C*ONH$_2$^{F}). * Chemical shifts and assignments were obtained from the HMQC spectrum. $C_{77}H_{116}N_5O_{35}P$ (1702.75, 1701.72), ESI ICR MS: *m*/*z* = 1700.73109 (calcd. 1700.71095) [M − H]$^-$, 849.85112 (calcd. 849.85152) [M − 2H]$^{2-}$. FAB MS: *m*/*z* = 1740.6 [M + K]$^+$, 1724.6 [M + Na]$^+$.

Ethyl (*E*)-4-Hydroxycinnamate:[19,30] R_f = 0.22 (CHCl$_3$). IR (KBr): $\tilde{\nu}$ = 3286, 1677, 1631, 1598, 1513, 1442, 1371, 1319, 1276, 1195, 1033, 977, 831, 518 cm^{-1}. UV (methanol): λ_{max} (ε) = 227 (32090), 301 (s) (52769), 310 nm (56851). ^{1}H NMR (200 MHz, CDCl$_3$): δ = 1.34 (t, *J* = 7.1 Hz, 3 H, OCH$_2$C*H*$_3$), 4.26 (q, *J* = 7.1 Hz, 2 H, OC*H*$_2$CH), 6.29 (d, *J*$_{2'\text{-}3'}$ = 15.9 Hz, 1 H, 2′-H), 6.86 (*J*$_{3\text{-}2}$ = 8.7 Hz, 2 H, 3-H, 5-H), 7.40 (*J*$_{2\text{-}3}$ = 8.7 Hz, 2 H, 2-H, 6-H,), 7.63 (d, *J*$_{3'\text{-}2'}$ = 15.9 Hz, 1 H, 3′-H). ^{13}C NMR (50 MHz, CDCl$_3$): δ = 14.4 (OCH$_2$*C*H$_3$), 60.8 (O*C*H$_2$CH$_3$), 115.3 (C-2′), 116.1 (C-3, C-5), 126.9 (C-1), 130.1 (C-2, C-6), 145.0 (C-3′), 158.3 (C-4), 168.2 (C-1′). $C_{11}H_{12}O_3$ (192.21, 192.08), FAB MS: *m*/*z* = 193.1 [M + H]$^+$.

Ethyl (*E*)-4-[2-(*tert*-Butoxycarbonylamino)ethoxy]cinnamate (20a): A solution of *tert*-butyl 2-bromoethylcarbamate[31] (1.17 g, 5.2 mmol) in DMF (10 mL) was added over 25 min to a suspension of ethyl 4-hydroxycinnamate (1.00 g, 5.2 mmol) and K$_2$CO$_3$ (0.72 g, 5.2 mmol) in DMF (10 mL). The mixture was stirred at 20 °C for 17 h, but no product formation was observed [TLC, CHCl$_3$/petroleum ether (light)/2-propanol, 10:10:1]. More K$_2$CO$_3$ (0.87 mg, 6.3 mmol) was then added, and the mixture was stirred at 20 °C for 3 d. TLC [CHCl$_3$/petroleum ether (light)/2-propanol, 10:10:1] indicated formation of **20a**, but the reaction was still not complete. Stirring was then continued at 60 °C for an additional 90 min (until starting material could no longer be observed). The reaction mixture was poured into water (170 mL) and extracted with ethyl acetate (3 × 70 mL). The solvent was removed from the combined organic extracts under reduced pressure, and the residue was purified by FC [CHCl$_3$/petroleum ether (light)/2-propanol, 10:10:1] to give a crude product that was further purified by FC (CHCl$_3$/petroleum ether/2-propanol, 10:15:1) to provide 1.7 g (97%) of pure **20a**. R_f = 0.37 [CHCl$_3$/petroleum ether (light)/2-propanol, 10:10:1]. M.p. 59 °C (after FC). IR (KBr): $\tilde{\nu}$ = 3280, 2975, 2929, 1708, 1693, 1631, 1600, 1542, 1511, 1363, 1251, 1172, 829 cm^{-1}. UV (methanol): λ_{max} (ε) = 225 (13221), 308 nm (26070). ^{1}H NMR (200 MHz, CDCl$_3$): δ = 1.32 (t, *J* = 7.1 Hz, 3 H, OCH$_2$C*H*$_3$), 1.45 [s, 9 H, (C*H*$_3$)$_3$C], 3.53 (dt, *J*$_{2\text{-}1}$ = 5.2 Hz, *J*$_{2\text{-}NH}$ = 5.6 Hz, 2 H, CH$_2$-2^{AE}), 4.04 (t, *J*$_{1\text{-}2}$ = 5.2 Hz, 2 H, CH$_2$-1^{AE}), 4.25 (q, *J* = 7.1 Hz, 2 H, OC*H*$_2$CH$_3$), 4.98 (broad s, 1 H, NH), 6.29 (d, *J*$_{2'\text{-}3'}$ = 15.9 Hz, 1 H, 2′-H), 6.88 (*J*$_{3\text{-}2}$ = 8.8 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.46 (*J*$_{2\text{-}3}$ = 8.8 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H), 7.63 (d, *J*$_{3'\text{-}2'}$ = 15.9 Hz, 1 H, 3′-H). ^{13}C NMR (50 MHz, CDCl$_3$): δ = 14.4 (OCH$_2$*C*H$_3$), 28.5 [(*C*H$_3$)$_3$C], 40.2 (C-2^{AE}), 60.4 (O*C*H$_2$CH$_3$), 67.4 (C-1^{AE}), 79.8 [(CH$_3$)$_3$*C*], 115.0 (C-3^{Ph}, C-5^{Ph}), 116.3 (C-2′), 127.8 (C-1^{Ph}), 129.9 (C-2^{Ph}, C-6^{Ph}), 144.3 (C-3′), 156.1 [O*C*(O)NH], 160.6 (C-4), 167.5

(C-1′). $C_{18}H_{25}NO_5$ (335.40, 335.17), ESI ICR MS: *m/z* = 693.33723 (calcd. 693.33554) [2 M + Na]$^+$, 671.35511 (calcd. 671.35434) [2 M + H]$^+$, 571.30328 (calcd. 571.30214) [2 M − $(CH_3)_2CCH_2$ − CO_2 + H]$^+$, 336.18102 (calcd. 336.18107) [M + H]$^+$, 280.11835 (calcd. 280.09127) [M − $(CH_3)_2CCH_2$ − CO_2 − H + 2 Na]$^+$.

(*E*)-4-[2-(*tert*-Butoxycarbonylamino)ethoxy]cinnamyl Alcohol (20b): A solution of BF_3·Et_2O (0.2 mL, 1.6 mmol) was added over 20 min to a cold (−78 °C) solution of **20a** (0.5 g, 1.5 mmol) in dry CH_2Cl_2 (7 mL). A solution of DIBAL-H (1 mol/L, 4.5 mL, 4.5 mmol) in CH_2Cl_2 (4.5 mL) was added at −78 °C, and the mixture was stirred at −78 °C for 2 h. Acetic acid (0.8 mL) and water (0.6 mL) were added to the reaction mixture. The temperature was allowed to increase to 20 °C, and the reaction mixture was extracted with diethyl ether. The ethereal extract was washed with aqueous $NaHCO_3$ and water, dried with Na_2SO_4, and filtered. The solvent was evaporated under reduced pressure to give 0.4 g of a white solid. Further purification by FC (cyclohexane/CH_2Cl_2/methanol, 10:10:1) gave 0.3 g (72%) of pure **20b**. R_f = 0.30 (cyclohexane/CH_2Cl_2/methanol, 10:10:1). M.p. 108 °C. IR (KBr): $\tilde{\nu}$ = 3565−3300, 2977, 2927, 1716, 1687, 1602, 1542, 1509, 1461, 1388, 1363, 1251, 1174, 1108, 1064, 971 cm^{-1}. UV (methanol): λ_{max} (ε) = 260 nm (19130). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.45 [s, 9 H, $(CH_3)_3C$], 3.52 (dt, $J_{2\text{-}1}$ = 5.1 Hz, $J_{2\text{-}NH}$ = 5.3 Hz, 2 H, CH_2-2^{AE}), 4.00 (t, $J_{1\text{-}2}$ = 5.1 Hz, 2 H, CH_2-1^{AE}), 4.28 (dd, $J_{1'\text{-}2'}$ = 5.8 Hz, $J_{1'\text{-}3'}$ = 1.4 Hz, 2 H, CH_2-1′), 5.02 (broad s, 1 H, NH), 6.23 (dt, $J_{2'\text{-}3'}$ = 15.8 Hz, $J_{2'\text{-}1'}$ = 5.8 Hz, 1 H, 2′-H), 6.54 (d, $J_{3'\text{-}2'}$ = 15.8 Hz, 1 H, 3′-H), 6.83 ($J_{3\text{-}2}$ = 8.8 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H,), 7.30 ($J_{2\text{-}3}$ = 8.8 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.5 [$(CH_3)_3C$], 40.3 (C-2^{AE}), 63.9 (C-1′), 67.3 (C-1^{AE}), 79.7 [$(CH_3)_3C$], 114.7 (C-3^{Ph}, C-5^{Ph}), 126.7 (C-2′), 127.8 (C-2^{Ph}, C-6^{Ph}), 130.0 (C-1^{Ph}), 130.8 (C-3′), 156.0 [OC(O)NH], 158.4 (C-4^{Ph}). $C_{16}H_{23}NO_4$ (293.36, 293.16), ESI ICR MS: *m/z* = 316.15195 (calcd. 316.15193) [M + Na]$^+$, 609.31464 (calcd. 609.31464) [2 M + Na]$^+$.

2-{(*E*)-4-[2-(*tert*-Butoxycarbonylamino)ethoxy]cinnamyl}-thiouronium Bromide (21a): A solution of PBr_3 (34 mg, 12 μL, 0.125 mmol) in dry CH_2Cl_2 (0.5 mL) was added over 15 min to a cooled (−10 °C) solution of **20b** (100 mg, 0.34 mmol) in dry CH_2Cl_2 (1.3 mL), and the reaction mixture was stirred at −10 °C for 25 min. Sat. aqueous $NaHCO_3$ (2 mL) was then added, the cooling bath was removed, and the mixture was extracted with diethyl ether. The ethereal extract was washed with water, dried with sodium sulfate, and filtered. The solvent was evaporated under reduced pressure and the crude product was dissolved in acetonitrile (2 mL). A solution of thiourea (23 mg, 0.27 mmol) in acetonitrile (0.5 mL) was then added to this solution. After a few minutes, a white precipitate formed in the clear reaction solution. The mixture was stirred for an additional 20 min. The product was then isolated by filtration and washed with acetonitrile. Drying in vacuum gave 93 mg (63% overall yield) of pure **21a**. IR (KBr): $\tilde{\nu}$ = 3600−3100 (broad, NH, NH_2), 1691, 1646, 1509, 1245, 1172 cm^{-1}. UV (water): λ_{max} (ε) = 266 nm (17297). UV (methanol): λ_{max} (ε) = 271 nm (27195). ^{1}H NMR (200 MHz, CD_3OD): δ = 1.44 [s, 9 H, $(CH_3)_3C$], 3.42 (t, $J_{2\text{-}1}$ = 5.8 Hz, 2 H, CH_2-2^{AE}), 4.00 (dd, $J_{1'\text{-}2'}$ = 7.4 Hz, $J_{1'\text{-}3'}$ = 1.1 Hz, 2 H, CH_2-1′), 4.01 (t, $J_{1\text{-}2}$ = 5.8 Hz, 2 H, CH_2-1^{AE}), 6.14 (dt, $J_{2'\text{-}3'}$ = 15.7 Hz, $J_{2'\text{-}1'}$ = 7.4 Hz, 1 H, 2′-H), 6.70 (d, $J_{3'\text{-}2'}$ = 15.7 Hz, 1 H, 3′-H), 6.90 ($J_{3\text{-}2}$ = 8.8 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.35 ($J_{2\text{-}3}$ = 8.8 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (50 MHz, CD_3OD): δ = 28.7 [$(CH_3)_3C$], 35.1 (C-1′), 41.0 (C-2^{AE}), 68.0 (C-1^{AE}), 80.3 [$(CH_3)_3C$], 115.8 (C-3^{Ph}, C-5^{Ph}), 119.8 (C-2′), 128.9 (C-2^{Ph}, C-6^{Ph}), 130.2 (C-1^{Ph}), 136.3 (C-3′), 158.5 [OC(O)NH], 160.5 (C-4^{Ph}), 172.4 [SC(NH)NH_2]. $C_{17}H_{25}N_3O_3S$ (base, 351.46, 351.16), FAB MS: *m/z* = 352.1 [M + H]$^+$, 276.1 [M − $SC(NH_2)_2$ + H]$^+$.

2-[(*E*)-4-(2-Ammonioethoxy)cinnamyl]thiouronium Bromide Chloride (21b): A mixture of **21a** (0.50 g, 1.16 mmol) in dry ethanol (15 mL) and HCl in ethanol (22%, 8 mL) was stirred at 20 °C for 2 h. The mixture was then poured into diethyl ether and the white precipitate that formed was collected, washed with ether, and dried in vacuo to give 0.35 g (82%) of **21b**. M.p. 194−196 °C. IR (KBr): $\tilde{\nu}$ = 3600−3100, 1649, 1603, 1509, 1248, 1016, 711 cm^{-1}. UV (methanol): λ_{max} (ε) = 265 nm (21583). ^{1}H NMR (200 MHz, CD_3OD): δ = 3.38 (t, $J_{2\text{-}1}$ = 4.9 Hz, 2 H, CH_2-2^{AE}), 4.02 (dd, $J_{1'\text{-}2'}$ = 7.1 Hz, $J_{1'\text{-}3'}$ = 1.1 Hz, 2 H, CH_2-1′), 4.25 (t, $J_{1\text{-}2}$ = 4.9 Hz, 2 H, CH_2-1^{AE}), 6.17 (dt, $J_{2'\text{-}3'}$ = 15.8 Hz, $J_{2'\text{-}1'}$ = 7.1 Hz, 1 H, 2′-H), 6.73 (d, $J_{3'\text{-}2'}$ = 15.8 Hz, 1 H, 3′-H), 7.00 ($J_{3\text{-}2}$ = 8.8 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.41 ($J_{2\text{-}3}$ = 8.8 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (50 MHz, CD_3OD): δ = 34.9 (C-1′), 40.3 (C-2^{AE}), 65.3 (C-1^{AE}), 115.9 (C-3^{Ph}, C-5^{Ph}), 120.4 (C-2′), 129.1 (C-2^{Ph}, C-6^{Ph}), 131.1 (C-1^{Ph}), 136.1 (C-3′), 159.6 (C-4^{Ph}), 172.3 [SC(NH)NH_2]. $C_{12}H_{17}N_3OS$ (base, 251.34, 251.11), FAB MS: *m/z* = 252.1 [M + H]$^+$, 176.1 [M − $SC(NH_2)_2$ + H]$^+$.

Treatment of 1 with 21a: A) Moenomycin A (**1**, 30 mg, 0.019 mmol), thiouronium salt **21a** (11.7 mg, 0.027 mmol), and sodium acetate (20 mg) in water (3 mL) were stirred under argon at 60 °C for 8 h. Progress of the reaction was monitored by TLC (RP_{18}, acetonitrile/H_2O, 7:10). No new product was observed. The mixture was then cooled to 20 °C, a further portion of thiouronium salt **21a** (11 mg, 0.019 mmol) and water (1 mL) were added, and stirring was continued at 60 °C for an additional 20 h. Formation of only one product was observed by TLC (RP_{18}, acetonitrile/H_2O, 7:10). The cold reaction mixture was directly transferred to the top of a FC (RP_{18}) column. The inorganic salts were removed by elution with water (50 mL) and the product was then eluted with acetonitrile/H_2O (7:10) to give 10 mg (45% yield, based on consumed **1**) of pure **22a**, with 11 mg of **1** recovered. B) Moenomycin A (**1**, 100 mg, 0.063 mmol), sodium acetate (50 mg), and thiouronium salt **21a** (85 mg, 0.23 mmol) in water (10 mL) were stirred under argon at 60 °C for 8 h. The mixture was then allowed to cool to 20 °C, and the pH was measured (pH = 5.38). The pH was adjusted to 7.25 by addition of sat. aqueous Na_2CO_3, and the reaction mixture was stirred at 60 °C for an additional 13 h. Progress of the reaction was monitored by TLC (RP_{18}, acetonitrile/H_2O, 6:10). Formation of two products (**23a** and **22a**) was observed. Kieselguhr was then added to the cold reaction solution, and water was lyophilized. The residue was transferred to the top of a FC (RP_{18}) column and the products were separated by elution with acetonitrile/H_2O, 6:10. 37.6 mg (37%) of **1**, 14.0 mg (12%) of **23a** and 17.4 mg (15%) of **22a** were obtained.

(*R*)-3-{[*N*-(1-{(*E*)-4-[2-(*tert*-Butoxycarbonylamino)ethoxy]-cinnamyl}-2,5-dioxocyclopentyl)-β-D-galactopyranuronamidosyl-(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 (22a): R_f = 0.28 (RP18, acetonitrile/H_2O, 7:10). UV (methanol): λ_{max} (ε) = 264 nm (13959). ^{1}H NMR (600 MHz, CD_3OD, ^{1}H,^{1}H COSY, HMQC, HMBC): δ = 0.98 (s, 6 H, CH_3-23^I, CH_3-24^I), 1.26 (s, 3 H, CH_3^F), 1.39 (m, 2 H, CH_2-9^I), 1.43 (d, *J* = 5.7 Hz, 3 H, CH_3-6^C), 1.46 (s, 9 H, $(CH_3)_3C$), 1.61 (s, 3 H, CH_3-20^I), 1.63 (s, 3 H, CH_3-21^I), 1.68 (s, 3 H, CH_3-19^I), 1.76 (s, 3 H, CH_3-25^I), 1.92 (m, 2 H, CH_2-10^I), 1.95 (s, 3 H, CH_3CONH^C or CH_3CONH^E), 2.01−2.19 (m, 11 H, CH_2-4^I, CH_2-5^I, CH_2-15^I,

CH_2-16^I, and 2.04 (s, CH_3CONH^E or CH_3CONH^C], 2.64 (m, 2 H, CH_2-1′), 2.71 (d, $J_{12\text{-}13}$ = 7.3 Hz, 2 H, CH_2-12^I), 2.79 (broad m, 1 H), 3.25−4.40 [broad signals of the sugar protons: 3.44 (t, $J_{2\text{-}1}$ = 5.2 Hz, CH_2-2^{AE}), 4.02 (bt, $J_{1\text{-}2}$ = 5.2 Hz, CH_2-1^{AE})] , 4.47, 4.57 (d, d, $J_{1\text{-}2}$ = 7.3 Hz, 1 H, 1 H, 1^C-H, 1^E-H), 4.49 (d, $J_{1\text{-}2}$ = 7.3 Hz, 1 H, 1^D-H), 4.68 (d, J = 8.4 Hz, 2 H, CH_2-22^I), 5.09−5.14 (m, 2 H, 3^F-H, 17^I-H), 5.15 (t, $J_{13\text{-}12}$ = 7.3 Hz, 1 H, 13^I-H), 5.31 (dt, $J_{6\text{-}7}$ = 15.7 Hz, $J_{6\text{-}5}$ = 6.8 Hz, 1 H, 6^I-H), 5.39 (d, $J_{7\text{-}6}$ = 15.7 Hz, 1 H, 7^I-H), 5.45 (broad signal, 1 H, 2^I-H), 5.95 (broad signal, 1 H, 1^F-H), 6.03 (dt, $J_{2'\text{-}3'}$ = 15.7 Hz, $J_{2'\text{-}1'}$ = 7.7 Hz, 1 H, 2′-H), 6.46 (d, $J_{3'\text{-}2'}$ = 15.7 Hz, 1 H, 3′-H), 6.89 (d, $J_{3\text{-}2}$ = 8.4 Hz, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.35 (d, $J_{2\text{-}3}$ = 8.4 Hz, 2 H, 2^{Ph}-H, 6^{Ph}-H). ^{13}C NMR (150 MHz, CD_3OD, APT, HMQC, HMBC): δ = 16.1 (C-21^I), 16.4 (CH_3^F), 17.7 (C-6^C), 17.8 (C-20^I), 23.4, 23.5 (*C*H_3CONH^C, *C*H_3CONH^E), 23.9 (C-25^I), 25.9 (C-19^I), 27.7 (C-16^I), 27.9 (C-23^I, C-24^I), 28.8 [(*C*H_3)$_3$C], 32.3 (C-10^I), 32.7 (C-5^I), 33.6 (C-4^I), 34.7 (broad signal), 35.9 (C-12^I), 36.4 (C-8^I), 36.9 (C-1′), 40.9 (C-15^I), 41.0 (C-2^{AE}), 42.9 (C-9^I), 56.1 (C-2^C), 56.8 (C-2^E), 62.8 (C-6^D), 67.0 (C-1^I), 68.0 (C-1^{AE}, C-1^A), 69.2, 70.0, 70.5, 70.7, 71.8, 72.2, 72.3, 72.6, 73.7, 74.1, 74.2, 74.3, 74.9, 75.1, 75.4, 76.1, 76.3 (C-3^F), 78.0, 78.1, 79.0 [(CH$_3$)$_3$*C*], 80.3, 81.2, 82.6 (C-4^E), 84.6 (C-4^C), 95.9 (C-1^F), 103.4 (C-1^C), 104.1, 104.5 (C-1^D, C-1^E), 104.7 (C-1^B), 109.3 (C-22^I), 115.7 (C-3^{Ph}, C-5^{Ph}), 119.1 (C-2′), 123.5 (C-13^I), 123.6 (C-2^I), 125.4 (C-17^I), 126.9 (C-6^I), 128.6, 128.8 (C-2^{Ph}, C-6^{Ph}), 131.1 (C-1^{Ph}), 131.8 (same HMBC as C-1^{Ph}), 132.2 (C-18^I), 133.9 (same HMBC as C-3′), 136.4 (C-3′), 137.3 (C-14^I), 140.7 (C-3^I), 141.5 (C-7^I), 151.1 (C-11^I), 158.5 [O*C*(O)NH), 159.3 (O*C*ONH_2^F), 159.6 (same HMBC as C-4^{Ph}), 160.1 (C-4^{Ph}), 170.4 (C-6^B), 170.9, 171.2, 173.8, 174.3 (CH_3*C*ONH^C and CH_3*C*ONH^E, C-6^F), 177.7 (CO-OH^H), 212.4, 213.2 (C-2^A, C-5^A). $C_{85}H_{129}N_6O_{37}P$ (1857.95, 1856.81), ESI ICR MS: *m*/*z* = 1855.83150 (calcd. 1855.80558) [M − H]$^-$, 927.40576 (calcd. 927.39884) [M − 2H]$^{2-}$.

(*R*)-3-[(*N*-{(*E*)-4-[4-(2-*tert*-Butoxycarbonylamino)ethoxyphenyl]-1-(3-carboxypropionyl)-3-butenyl}-β-D-galactopyranuronamidosyl-(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 (23a): R_f = 0.38 (RP_{18}, acetonitrile/H_2O, 6:10). UV (methanol): λ_{max} (ε) = 262 nm (14886). ^{1}H NMR (600 MHz, CD_3OD, ^{1}H,^{1}H COSY, HMQC, HMBC): δ = 0.98 (s, 6 H, CH_3-23^I, CH_3-24^I), 1.26 (s, 3 H, CH_3^F), 1.39 (m, 5 H, CH_2-9^I, CH_3-6^C), 1.46 (s, 9 H, (C*H*$_3$)$_3$C), 1.61 (s, 3 H, CH_3-20^I), 1.63 (s, 3 H, CH_3-21^I), 1.68 (s, 3 H, CH_3-19^I), 1.76 (s, 3 H, CH_3-25^I), 1.92 (m, 2 H, CH_2-10^I), 2.00−2.20 (m, 16 H, CH_2-4^I, CH_2-5^I, CH_2-15^I, CH_2-16^I, C*H*$_3$CONH^C, C*H*$_3$CONH^E), 2.39−2.49 (m, 2 H, CH_2-3^A, CH_2*-3^A), 2.49−2.56 (m, 0.5 H, 2′-H$_a$), 2.57−2.65 (m, 0.5 H, 2′-H$_a$*), 2.71 (d, 2 H, CH_2-12^I, $J_{12\text{-}13}$ = 7.3 Hz), 2.73−2.84 (m, 2 H, 2^A-H$_a$, 2^A-H$_a$*, 2′-H$_b$, 2′-H$_b$*), 2.84−2.95 (m, 1 H, 2^A-H$_b$, 2^A-H$_b$*), 3.22−4.52 [broad signals of the sugar protons: 3.43 (broad, CH_2-2^{AE}), 3.99 (broad, CH_2-1^{AE}), 3.66 (2^F-H), 4.12 (1^I-H$_b$), 4.23 (1^I-H$_a$), 4.51 (1^D-H)], 4.54−4.63 (broad, 1′-H), 4.68 (d, J = 8.4 Hz, 2 H, CH_2-22^I), 5.07−5.13 (m, 2 H, 3^F-H, 17^I-H), 5.15 (t, $J_{13\text{-}12}$ = 7.3 Hz, 1 H, 13^I-H), 5.31 (dt, $J_{6\text{-}7}$ = 15.1 Hz, $J_{6\text{-}5}$ = 6.4 Hz, 1 H, 6^I-H,), 5.38 (d, $J_{7\text{-}6}$ = 15.1 Hz, 1 H, 7^I-H), 5.45 (bt, 1 H, 2^I-H), 5.96 (broad signal, 1 H, 1^F-H), 6.04 (dt, $J_{3'\text{-}4'}$ = 15.7 Hz, $J_{3'\text{-}2'}$ = 7.7 Hz, 0.5 H, 3′-H*), 6.11 (dt, $J_{3'\text{-}4'}$ = 15.7 Hz, $J_{3'\text{-}2'}$ = 7.7 Hz, 0.5 H, 3′-H), 6.44 (d, $J_{4'\text{-}3'}$ = 15.2 Hz, 0.5 H, 4′-H), 6.52 (d, $J_{4'\text{-}3'}$ = 15.2 Hz, 0.5 H, 4′-H*), 6.87 (d, $J_{3\text{-}2}$ = 7.8 Hz, 2 H, 3^{Ar}-H, 5^{Ar}-H, 3^{Ar}-H*, 5^{Ar}-H*), [2 H, 7.33 (d, $J_{2\text{-}3}$ = 7.8 Hz, 2^{Ar}-H*, 6^{Ar}-H*), 7.35 (d, $J_{2\text{-}3}$ = 7.8 Hz, 2^{Ar}-H, 6^{Ar}-H)]. ^{13}C NMR (150 MHz, CD_3OD, APT, HMQC, HMBC): δ = 16.1 (C-21^I), 16.3 (CH_3^F), 17.8, 17.9 (C-20^I, C-6^C), 23.4, 23.5 (*C*H_3CONH^C, *C*H_3CONH^E), 24.0 (C-25^I), 25.9 (C-19^I), 27.7 (C-16^I), 27.8 (C-23^I, C-24^I), 28.8 [(*C*H$_3$)$_3$C), 32.3 (C-10^I), 32.5 (C-3^A), 32.7 (C-5^I), 33.5 (C-4^I), 34.8, 35.0 (C-2′)*, 35.9 (C-12^I), 36.4 (C-8^I), 40.9 (C-15^I), 41.0 (C-2^{AE}), 42.9 (C-9^I), 55.9, 55.9 (C-2^E)*, 56.9, 57.1 (C-2^C)*, 59.9, 60.3 (C-1′)*, 62.7 (C-6^D), 66.9 (C-1^I), 67.9 (C-1^{AE}), 69.2, 69.8, 70.0, 70.1, 70.8, 70.9, 71.7, 72.1, 72.2, 72.6, 73.8 (C-5^F), 74.1, 74.4, 75.0, 75.1, 76.2 (C-3^F), 77.9, 78.1, 79.1 (C-2^F), 80.2 (C-13^{Ar}), 81.0 (C-2 ^{H}), 82.4 (C-4^E), 84.5, 84.8 (C-4^C)*, 95.9 (C-1^F), 103.1, 103.3 (C-1^C)*, 104.2, 104.4 (C-1^E, C-1^D), 104.6, 104.7 (C-1^B)*, 109.3 (C-22^I), 115.6 (C-3^{Ar}, C-5^{Ar}), 123.5 (C-13^I, C-2^I), 123.9, 124.1 (C-3′)*, 125.4 (C-17^I), 126.9 (C-6^I), 128.6 (C-2^{Ar}, C-6^{Ar}), 131.8 (C-1^{Ar}), 132.2 (C-18^I), 133.8, 133.9 (C-4′)*, 137.3 (C-14^I), 140.8 (C-3^I), 141.5 (C-7^I), 151.1 (C-11^I), 158.5 (NH*C*(O)O), 159.3 (O*C*ONH_2^F), 159.6 (C-4^{Ar}), 170.7, 171.1 (*C*ONH^B)*, 173.9, 174.2 (CH_3*C*ONH^C, CH_3*C*ONH^E), 174.3 (*C*ONH_2^F), 177.7 (C-1^H), 180.8 (*C*O_2H^A), 210.1 (C-1^A). * Signals from the second diastereomer. $C_{85}H_{131}N_6O_{38}P$ (1875.96, 1874.82), ESI ICR MS: *m*/*z* = 947.39030 (calcd. 947.39517) [M + Na − 3 H]$^{2-}$, 936.40002 (calcd. 936.40412) [M − 2 H]$^{2-}$, 623.93157 (calcd. 623.93345) [M − 3 H]$^{3-}$.

Treatment of 1 with 21b: A) Moenomycin A (**1**, 100 mg, 0.063 mmol), sodium acetate (100 mg), and thiouronium salt **21b** (42 mg, 0.114 mmol) in water (3 mL) were stirred under argon at 60 °C for 20 h. More thiouronium salt **21b** (30 mg, 0.081 mmol), sodium acetate (230 mg), and water (0.8 mL) were then added to the reaction mixture, and stirring and heating were continued for an additional 25 h. A further amount of compound **21b** (30 mg, 0.081 mmol, total 102 mg, 0.276 mmol), sodium acetate (100 mg, total 430 mg), and water (0.4 mL, total 4.2 mL) were added, and the mixture was stirred at 60 °C for 21 h (altogether 66 h). Progress of the reaction was continuously monitored by TLC (RP_{18}, acetonitrile/H_2O, 7:10) and it was found that two products had been formed but the reaction was still not complete. The reaction mixture was allowed to cool to ambient temperature. Inorganic salts were removed by ultrafiltration (4 × 50 mL water). Water was lyophilized, and the crude products were purified twice by FC (RP_{18}, acetonitrile/H_2O, 7:10). After acetonitrile evaporation under reduced pressure and lyophilization, 34 mg (15%) of **22b** and 18 mg (8%) of **23b** were obtained. B) A solution of moenomycin A (**1**, 150 mg, 0.095 mmol), sodium acetate (50 mg, 0.609 mmol), and thiouronium salt **21b** (49 mg, 0.133 mmol) in water (15 mL, pH = 5.74) was stirred under argon at 60 °C for 47 h and then at 20 °C overnight. Progress of the reaction was monitored by TLC (RP_{18}, acetonitrile/H_2O, 7:10, R_f = 0.23, $R_f^{\text{moenomycin A}}$ = 0.47) (**22b** could be not detected and separated by normal phase chromatography) and MALDI TOF MS. Kieselguhr was then added to the cold reaction mixture, and water was lyophilized. The residue was transferred to the top of a FC column (RP_{18}). Elution with acetonitrile/H_2O (6:10) gave (after lyophilization of solvents) 71 mg (43%, 81% based on consumed **1**) of pure **22b**, 121 mg of **1**, and inorganic salts. C) A solution of moenomycin A (**1**) (100 mg, 0.063 mmol), sodium acetate (80 mg), and thiouronium salt **21b** (73 mg, 0.197 mmol) in water (10 mL, pH = 7.30) was stirred under argon at 60 °C for 7 h. Progress of the reaction was monitored by TLC (RP18, acetonitrile/H_2O, 6:10) and formation only of product **22b** was observed (R_f = 0.26). After the reaction mixture had cooled, the pH was measured (pH = 5.56). The pH of the reaction mixture was then adjusted to 7.71 by addition of sat. aq. Na_2CO_3 and the mixture was stirred at 60 °C for an additional 25 h. After this period, TLC showed only one product (**23b**), which differed from that first detected (R_f = 0.37). The cold reaction mixture was poured into an ultrafiltration cell (50 mL) and inorganic salts were

removed by elution with water (120 mL). Kieselguhr was then added to the residue, and the solvent was lyophilized. The Kieselguhr with the adsorbed substances was transferred to the top of a FC column (RP18). Elution with acetonitrile/H_2O (6:10) gave 42 mg of crude **23b**, with 33 mg of **1** recovered. Repeated FC (RP18, acetonitrile/H_2O, 6:10) provided 24 mg (22%) of pure **23b**.

(*R*)-3-[(*N*-{(*E*)-4-[4-(2-Aminoethoxy)phenyl]-1-(3-carboxypropionyl)-3-butenyl}-β-D-galactopyranuronamidosyl-(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 (23b): R_f = 0.33 (RP_{18}, acetonitrile/H_2O, 7:10). UV (methanol): λ_{max} (ε) = 262 nm (14704). ^{1}H NMR (600 MHz, CD_3OD, ^{1}H,^{1}H COSY, HMBC, HMQC): δ = 0.96 (s, 6 H, CH_3-23^I, CH_3-24^I), 1.25 (s, 3 H, CH_3^F), 1.29 (d, *J* = 6.3 Hz, 3 H, CH_3-6^C), 1.35 (m, 2 H, CH_2-9^I), 1.59 (s, 3 H, CH_3-20^I), 1.61 (s, 3 H, CH_3-21^I), 1.67 (s, 3 H, CH_3-19^I), 1.74 (s, 3 H, CH_3-25^I) 1.89 (m, 2 H, CH_2-10^I), 2.00−2.20 (m, 14 H, CH_2-4^I, CH_2-5^I, CH_2-15^I, CH_2-16^I, and 2.06, 2.07 (s, s, C*H*$_3$CONH^C, C*H*$_3$CONH^E), 2.40−2.48 (m, 2 H, CH_2-3^A, CH_2*-3^A), 2.60−2.66 (m, 0.5 H, 2′-H*$_a$), 2.69 (d, $J_{12\text{-}13}$ = 7.3 Hz, 2 H, CH_2-12^I), 2.72−2.77 (m, 1 H, 2′-H$_{a,b}$), 2.79−2.94 (m, 2.5 H, 2^A-H$_{a,b}$, 2^A-H$_{a,b}$*, 2′-H$_b$*), 3.20−4.80 [broad signals of the sugar protons: 3.43 (CH_2-2^{AE}), 4.28 (CH_2-1^{AE}), 4.50, 4.69* (CH-1′)], 5.07−5.14 (m, 13^I-H, 17^I-H), 5.27 (dt, $J_{6\text{-}7}$ = 15.5 Hz, $J_{6\text{-}5}$ = 6.4 Hz, 1 H, 6^I-H), 5.34 (d, $J_{7\text{-}6}$ = 15.5 Hz, 1 H, 7^I-H), 5.42 (broad signal, 1 H, 2^I-H), 5.88 (m, 1 H, 1^F-H), 5.96 (dt, $J_{3'\text{-}4'}$ = 15.5 Hz, $J_{3'\text{-}2'}$ = 7.6 Hz, 0.5 H, 3′-H*), 6.09 (dt, $J_{3'\text{-}4'}$ = 15.5 Hz, $J_{3'\text{-}2'}$ = 7.6 Hz, 0.5 H, 3′-H), 6.46 (d, $J_{4'\text{-}3'}$ = 15.5 Hz, 0.5 H, 4′-H), 6.48 (d, $J_{4'\text{-}3'}$ = 15.5 Hz, 0.5 H, 4′-H*), 6.99 (d, $J_{3\text{-}2}$ = 8.9 Hz, 1 H, 3^{Ar}-H, 5^{Ar}-H), 7.01 (d, $J_{3\text{-}2}$ = 8.9 Hz, 1 H, 3^{Ar}-H*, 5^{Ar}-H*), 7.34 (d, $J_{2\text{-}3}$ = 8.9 Hz, 1 H, 2^{Ar}-H, 6^{Ar}-H), 7.39 (d, $J_{2\text{-}3}$ = 8.9 Hz, 1 H, 2^{Ar}-H*, 6^{Ar}-H*). ^{13}C NMR (150 MHz, CD_3OD, HMBC, HMQC): δ = 16.1 (C-21^I), 16.3 (CH_3^F), 17.8 (C-20^I), 18.0, 18.2 (C-6^C)*, 23.5 (*C*H$_3$CONH^C, *C*H$_3$CONH^E), 23.9 (C-25^I), 25.9 (C-19^I), 27.4 (C-16^I), 27.8 (C-23^I, C-24^I), 32.0 (C-10^I), 32.2 (C-3^A), 32.5 (C-5^I), 33.3 (C-4^I), 34.2, 34.6 (C-2′)*, 35.9 (C-12^I), 36.3 (C-8^I), 40.3 (C-2^{AE}), 40.6 (C-15^I), 42.7 (C-9^I), 56.7 (C-2^C, C-2^E), 59.6, 59.9 (C-1′)*, 62.4 (C-6^D), 65.6 (C-1^{AE}), 66.9−81.1 (broad overlapping signals of the sugar carbon atoms), 95.7 (C-1^F), 102.7−103.4 (broad signals, C-1^C, C-1^B, C-1^E, C-1^D), 109.4 (C-22^I), 115.8, 116.1 (C-3^{Ar}, C-5^{Ar})*, 122.9 (C-2^I), 123.4 (C-13^I), 123.6 (C-3′), 125.2 (C-17^I), 126.9 (C-6^I), 128.6, 128.7 (C-2^{Ar}, C-6^{Ar})*, 132.2 (C-1^{Ar}), 132.7 (C-8^I), 134.1 (C-4′), 137.3 (C-14^I), 141.4 (C-7^I, C-3^I), 151.2 (C-11^I), 158.8 (C-4^{Ar}), 159.2 (O*C*ONH$_2^F$), 170.1, 170.8 (*C*ONH^B)*, 174.3 (*C*ONH$_2^F$), 174.4, 174.5 (CH$_3$*C*ONH^C, CH$_3$*C*ONH^E), 177.6 (C-1 ^{H}), 181.1 (*C*O$_2$H^A), 201.4 (C-1^A). * Signals of the second diastereomer. $C_{80}H_{123}N_6O_{36}P$ (1775.84, 1774.77), ESI ICR MS: *m*/*z* = 886.37477 (calcd. 886.37791) [M − 2 H]$^{2-}$, 590.58070 (calcd. 590.58264) [M − 3 H]$^{3-}$.

(*R*)-3-{[*N*-(1-{(*E*)-4-[2-Aminoethoxy]cinnamyl}-2,5-dioxocyclopentyl)-β-D-galactopyranuronamidosyl-(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 (22b): R_f = 0.23 (RP_{18}, acetonitrile/H_2O, 7:10). UV (methanol): λ_{max} (ε) = 263 nm (27344). ^{1}H NMR (600 MHz, CD_3OD/[D$_6$]DMSO, 3:1, ^{1} H,^{1}H COSY, HMQC, HMBC): δ = 0.98 (s, 6 H, CH_3-23^I, CH_3-24^I), 1.24 (s, 3 H, CH_3^F), 1.40 (broad signal, 5 H, CH_3-6^C, CH_2-9^I), 1.62 (s, 3 H, CH_3-20^I), 1.63 (s, 3 H, CH_3-21^I), 1.69 (s, 3 H, CH_3-19^I), 1.75 (s, 3 H, CH_3-25^I), 1.92 (broad signal, 5 H, C*H*$_3$CONH^C or C*H*$_3$CONH^E and CH_2-10^I), 2.00−2.07 (m, 5 H, H$_a$-15^I, H$_a$-16^I and 2.03 (s, C*H*$_3$CONH^E or C*H*$_3$CONH^C)], 2.08−2.20 (m, 6 H, CH_2-4^I, CH_2-5^I, 15^I-H$_b$, 16^I-H$_b$), 2.58−2.68 (broad signal, hidden by DMSO signal, CH_2-1′), 2.71 (d, $J_{12\text{-}13}$ = 7.1 Hz, 2 H, CH_2-12^I), 2.80 (broad d, 2 H, CH_2-3^A or CH_2-4^A), 2.90 (broad d, 2 H, CH_2-4^A or CH_2-3^A), 3.26 (broad signal, 1 H, 2^D-H), 3.25−4.60 [broad overlapping signals of the sugar protons: 3.33 (4^D-H), 3.43 (broad signal, CH_2-2^{AE}), 3.66 (2^F-H, 5^D-H), 3.89 (6^D-H$_a$), 3.97 (broad signal, 1 H, 2^H-H), 4.03−4.19 (broad signal, 3 H, 1^I-H$_a$, 6^D-H$_b$, 3^H-H$_a$), 4.21 (broad signal, 1 H, 1^I-H$_b$), 4.30 (broad signal, 3^H-H$_b$, CH_2-1^{AE}), 4.46 (broad signal, 3 H, 1^B-H, 1^C-H, 1^E-H)], 5.07 (d, $J_{3\text{-}2}$ = 10.0 Hz, 1 H, 3^F-H), 5.12 (t, $J_{17\text{-}16}$ = 6.9 Hz, 1 H, 17^I-H), 5.16 (t, $J_{13\text{-}12}$ = 7.1 Hz, 1 H, 13^I-H), 5.30 (dt, $J_{6\text{-}7}$ = 15.2 Hz, $J_{6\text{-}5}$ = 5.7 Hz, 1 H, 6^I-H), 5.38 (d, $J_{7\text{-}6}$ = 15.2 Hz, 1 H, 7^I-H), 5.45 (broad signal, 1 H, 2^I-H), 5.90 (broad signal, 1 H, 1^F-H), 6.10 (m, 1 H, 2′-H), 6.47 (d, $J_{3'\text{-}2'}$ = 14.8 Hz, 1 H, 3′-H), 7.04 (d, $J_{3\text{-}2}$ = 8.1 Hz, 2 H, 3^{Ar}-H, 5^{Ar}-H), 7.40 (d, $J_{2\text{-}3}$ = 8.1 Hz, 2 H, 2^{Ar}-H, 6^{Ar}-H). ^{13}C NMR (150 MHz, CD_3OD/[D$_6$]DMSO, 3:1, HMQC, HMBC): δ = 13.3 (impurities from RP_{18} material), 15.4 (CH_3-21^I), 15.9 (CH_3^F), 17.1 (CH_3-20^I), 17.2 (C-6^C), 19.89 (impurities from RP_{18} material), 22.8 (*C*H$_3$CONH^C), 22.9 (*C*H$_3$CONH^E), 23.3 (C-25^I), 25.3 (C-19^I), 26.8 (C-16^I), 27.1 (C-23^I, C-24^I), 29.7 (impurities from RP_{18} material), 31.4 (C-10^I), 31.8 (C-5^I), 32.7 (C-4^I), 34.5 (broad, C-3^A, C-4^A), 35.1 (C-12^I), 35.6 (C-8^I, C-1′ (HMBC 2′, 3′-H), 39.5 (C-2^{AE}), 39.9 (C-15^I), 41.9 (C-9^I), 55.5 (C-2^E), 55.9 (C-2^C), 61.8 (C-6^D), 64.9 (C-1^{AE}), 66.1 (C-1^I), 66.9, 68.2, 68.8, 69.5 (C-3 ^{H}), 70.7, 71.2, 71.6, 72.8 (C-2^F), 73.1, 73.3, 73.4, 74.1, 74.6, 75.0 (C-3^F), 77.3, 77.9 (C-2^F), 80.3 (C-2^H), 81.6 (C-4^E.), 83.8 (C-4^C), 94.9 (C-1^F), 102.4, 102.8, 103.6, 103.6 (C-1^C, C-1^E, C-1^B, C-1^D), 108.6 (C-22^I), 115.3 (C-3^{Ar}, C-5^{Ar}), 119.1 (C-2′), 122.6 (C-13^I), 122.9 (C-2^I), 124.5 (C-17^I), 126.1 (C-6^I), 128.1 (C-2, 6^{Ar}), 130.7 (C-1^{Ar}), 131.3 (C-18^I), 135.1 (C-3′), 136.5 (C-14^I), 139.52 (C-3^I), 140.5 (C-7^I), 150.2 (C-11^I), 157.9 (O*C*ONH$_2^F$), 158.5 (C-4^{Ar}), 169.4 (*C*ONH^B), 172.3, 172.6 (CH$_3$*C*ONH^C, CH$_3$*C*ONH^E), 173.3 (*C*ONH$_2$ ^{F}), 176.0 (small signal), 176.3 (C-1 ^{H}), 210.9, 211.5 (C-2^A, C-5^A). $C_{80}H_{121}N_6O_{35}P$ (1757.83, 1756.76), ESI ICR MS: *m*/*z* = 1755.73669 (calcd. 1755.75377) [M − H]$^-$, 877.37116 (calcd. 877.37325) [M − 2 H]$^{2-}$.

(*R*)-3-[(*N*-{(*E*)-4-[4-(2-{[2-({2-[3′,6′-Bis(ethylamino)-2′,7′-dimethyl-3-oxospiro[1*H*-isoindole-1,9′-[9*H*]xanthen]-2(3 *H*)-yl]ethyl}amino)-3,4-dioxo-1-cyclobuten-1-yl]amino}ethoxy)phenyl]-1-[3-(methoxycarbonyl)propionyl]-3-butenyl}-β-D-galactopyranuronamidosyl-(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 (25): A solution of **24** (15 mg, 0.025 mmol) in EtOH (0.5 mL) was added to a solution of **22b** (30 mg, 0.017 mmol) in methanol (7 mL) and Et_3N (1 mL). The heterogeneous mixture was stirred at 20 °C. After 18 h, the mixture became homogeneous, and it was stirred at 20 °C for an additional 72 h. Progress of the reaction was continuously monitored by TLC (1-propanol/H_2O, 7:2). TLC indicated the formation of two diastereoisomers of **25**. Kieselguhr was added to the cooled solution and the solvents were evaporated under reduced pressure. The residue was transferred to the top of a FC column. Elution with 1-propanol/H_2O (7:1.5), solvent evaporation, and lyophilization provided 22 mg (56%) of pure **25** (two diastereomers). R_f * = 0.62, R_f = 0.58 (1-propanol/H_2O, 7:2). ^{1}H NMR (600 MHz, CD_3OD/[D$_6$]DMSO, 5:1, ^{1}H,^{1}H COSY, HMQC, HMBC): δ = 1.01 (s, 6 H, CH_3-23^I, CH_3-24^I), 1.26 (s, 3 H, CH_3^F), 1.32, 1.33 (broad signals,

6 H, 2 NHCH$_2$CH_3^{R6G}), 1.37−1.47 (broad signal, 5 H, CH$_2$-9^I, CH$_3$-6^C), 1.64 (s, 6 H, CH$_3$-20^I, CH$_3$-21^I), 1.71 (s, 3 H, CH$_3$-19^I), 1.79 (s, 3 H, CH$_3$-25^I), 1.92, 1.93 (s, 6 H, 2′^{R6G}-CH_3, 7′^{R6G}-CH_3), 1.92−1.98 (broad signal, 2 H, CH$_2$-10^I), 2.02−2.10 (broad signals, 8 H, CH_3CONH^C, CH_3CONH^E, 15^I-H$_a$, 16^I-H$_a$), 2.11−2.17 (broad signal, 6 H, CH$_2$-4^I, CH$_2$-5^I, 15^I-H$_b$, 16^I-H$_b$), 2.18−2.25 (acetone), 2.49−2.64 (broad m, 3 H, 2′-H$_a$, CH$_2$*-3^A, CH$_2$-3^A, 2′-H*$_a$), 2.70−2.80 (broad signal, 3 H, CH$_2$-12^I, 2′-H$_b$, 2′-H*$_b$), 2.90−3.02 (broad signal, 2 H, CH$_2$-2^A, CH$_2$*-2^A), 3.20−4.60 [broad overlapping signals of the sugar protons with 3.24 (broad signal, 2 NHCH_2CH$_3^{R6G}$), 3.67 (broad s, OCH$_3^A$), 4.48 (broad signal, 1′-H)], 5.14 (broad m, 2 H, 17^I-H, 3^F-H), 5.18 (broad signal, 1 H, 13^I-H), 5.36 (broad signal, 1 H, 6^I-H), 5.43 (d, 7^I-H, $J_{7\text{-}6}$ = 17.8 Hz), 5.46 (broad signal, 1 H, 2^I-H), 5.95 (broad signal, 1 H, 1^F-H), 6.05 (broad signal, 0.5 H, 3′-H), 6.14 (broad signal, 0.5 H, 3′-H*), 6.18 (s, 2 H, 1′^{R6G}-H, 8′^{R6G}-H), 6.37 (s, 2 H, 4′^{R6G}-H, 5′^{R6G}-H), 6.44 (d, $J_{4'\text{-}3'}$ = 15.5 Hz, 0.5 H, 4′-H*), 6.55 (d, $J_{4'\text{-}3'}$ = 15.5 Hz, 0.5 H, 4′-H), 6.93 (broad signal, 2 H, 3^{Ph}-H, 5^{Ph}-H), 7.07 (broad signals, 1 H, 7^{R6G}-H), 7.36 (broad signal, 2 H, 2^{Ph}-H, 6^{Ph}-H), 7.58 (broad signal, 2 H, 5^{R6G}-H, 6^{R6G}-H), 7.92 (broad signal, 1 H, 4^{R6G}-H). ^{13}C NMR (150 MHz, CD$_3$OD/[D$_6$]DMSO, 5:1, HMQC, HMBC): δ = 14.1 (NHCH$_2$CH_3^{R6G}), 15.5 (C-21^I), 15.9 (CH$_3^F$), 16.6 (2′-CH_3^{R6G}, 7′-CH_3^{R6G}), 17.2 (C-20^I, C-6^C), 22.9 (CH_3CONH^C, CH_3CONH^E), 23.4 (C-25^I), 25.3 (C-19^I), 26.8 (C-16^I), 27.2 (C-23^I, C-24^I), 27.7, 27.7 (C-3^A)*, 30.1 (acetone), 31.5 (C-10^I), 31.9 (C-5^I, C-2′ (HMBC), 32.6 (C-4^I), 34.0, 34.1 (C-2^A)*, 34.3 (impurities), 35.1 (C-12^I), 35.6 (C-8^I), 38.2 (NHCH_2CH$_3^{R6G}$), 39.9 (C-15^I), 41.8 (C-2^{AE}), 41.9 (C-9^I), 43.8 (C-1^{DAE}), 51.4, 51.5 (OCH_3^A)*, 55.2 (C-2^E), 56.0 (C-2^C), 58.9, 59.2 (C-1′)*, 61.9 (C-6^D), 66.3 (C-9′^{R6G}), 68.0, 69.0 (broad signal, C-3 ^{H}), C-70.1, 70.9, 71.1, 71.2, 71.6, 71.7, 72.8, 73.1, 73.3, 73.6, 74.2, 75.2, 75.3, 77.2, 77.3 (broad unassigned signals of the sugar carbon atoms), 77.9 (broad C-2^F), 81.8 (C-4^E), 83.7, 83.9 (C-2 ^{H}, C-4^C), 94.9 (C-1^F), 96.5 (C-4′^{R6G}, C-5′^{R6G}), 102.4, 103.1, 103.7 (broad, C-1^C, C-1^B, C-1^E, C-1^D), 104.9 (C-8′a^{R6G}, C-9′a^{R6G}), 108.6 (C-22^I), 115.1 (C-3^{Ph}, C-5^{Ph}), 119.1 (C-2′^{R6G}, C-7′^{R6G}), 122.6 (C-13^I), 122.9 (C-4^{R6G}), 123.4, 123.4 (C-2^I), 124.3 (C-7^{R6G}), 124.6 (C-17^I), 126.1 (C-6^I), 127.9 (C-2^{Ph}, C-6^{Ph}), 128.1 (C-13^I), 128.8 (C-5^{R6G}), 131.1 (C-3a^{R6G}), 131.4 (C-18^I), 132.7 (C-1^{Ph}), 133.1 (C-4′), 133.4 (C-6^{R6G}), 136.5 (C-14^I), 140.6 (C-7^I), 148.5 (C-3′^{R6G}, C-6′^{R6G}), 150.1 (C-11^I), 152.3 (C-4′a^{R6G}, C-10′a^{R6G}), 154.4 (C-7a^{R6G}), 157.9 (OCONH$_2^F$), 158.5 (C-4^{Ph}), 168.3, 168.6 (CONH^B)*, 169.6 (CONH^{R6G}), 169.8, 170.1 (C-1^{SA}, C-2^{SA}), 172.3 (CH$_3$CONH^C, CH$_3$CONH^E), 173.2 (CONH^F), 173.8 (CO$_2$H^A), 182.8, 183.3 (C-3^{SA}, C-4^{SA}), 207.8 (acetone), 208.4, 208.5 (C-1^A)*. * Signals of the second diastereomer. $C_{113}H_{155}N_{10}O_{40}P$ (2324.49, 2323.01), ESI ICR MS: *m/z* = 1160.50236 (calcd. 1160.49971) [M − 2H]$^{2-}$, 773.33291 (calcd. 773.33071) [M − 3 H]$^{3-}$. Fluorescence spectrum [methanol/H$_2$O, 1:1 (4 mL) + 0.2% TFA (1 mL), 0.22 mg/5 mL]: excitation (550 nm): λ_{max} = 527 nm, λ_{max} = 350 nm, emission (527 nm): λ_{max} = 555 nm.

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