Synthesis of a Doubly Labelled Concanamycin Derivative for ATPase Binding Studies[‡]

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The synthesis of a doubly labelled concanamycin derivative for binding studies with V- and P-type ATPases is described. The starting point was 21-deoxyconcanolide A (**6**), which was generated from concanamycin A (**1**) in three steps and which exhibited the full ATPase inhibitor activity, with the advantage of a stability better than that of **1**. Through use of a suitable protecting group for **6**, the carbene-generating diazirine residue and ^{125}I were introduced regio- and stereoselectively. The inhibitory efficacy of the resulting 23-iodo(^{125}I)-9-*O*-[*p*-(trifluoroethyldiazirinyl)benzoyl]-21,23-dideoxyconcanolide A (**11b**) turned out to be high enough for labelling studies. Photoaffinity labelling experiments clearly showed that **11b** is a suitable derivative with which to determine the binding site of concanamycin-like compounds in different ATPases.

Introduction

Asymmetric plecomacrolides[2] with 18- and 16-membered macrolactone skeletons, such as concanamycins A (**1**; Figure 1) and C (**2**) and the bafilomycins, respectively, have been shown to be the most specific inhibitors (nanomolar concentrations) of V-type (vacuolar) ATPases.[3,4] Some P-type ATPases are also inhibited by these plecomacrolides (micromolar concentrations), whereas the F-type ATPases are not affected. The fact that the V-type ATPases are involved in the process of osteoclast bone resorption[5] made the plecomacrolides interesting compounds for pharmacological studies.[6] However, concanamycin A (**1**), the most potent inhibitor, exhibits an undesirably high toxicity,[7,8] which reduces its prospects for pharmaceutical use. Nevertheless, the concanamycins are important tools for the study of the physiological and biochemical properties of V-type and P-type ATPases.[9]

Despite the intensive use of plecomacrolides in biochemical research, the exact molecular mechanism of inhibition and the binding site within the enzyme complex is still unknown.[10,11] We therefore set out to synthesise a concanamycin derivative containing both a photoreactive group and an additional moiety acting as a tracer group, to enable the inhibitor binding site to be detected after successful labelling. The first encouraging results from structure/activity studies[4] indicated that the incorporation of functional groups without serious effects on the inhibitory potential of the plecomacrolide should be possible. In order to prepare suitable semisynthetic concanamycin derivatives, we studied the reactivities of different functional groups in the antibiotics by means of *O*-acylation, *O*-alkylation and nucleophilic substitution.[1,12–14] The derivatives were tested for their ATPase inhibitory activity. As a result, the pharmacophore of the plecomacrolide was identified[12,15] and specific labelling of the V-type ATPase of *Manduca Sexta* was achieved.

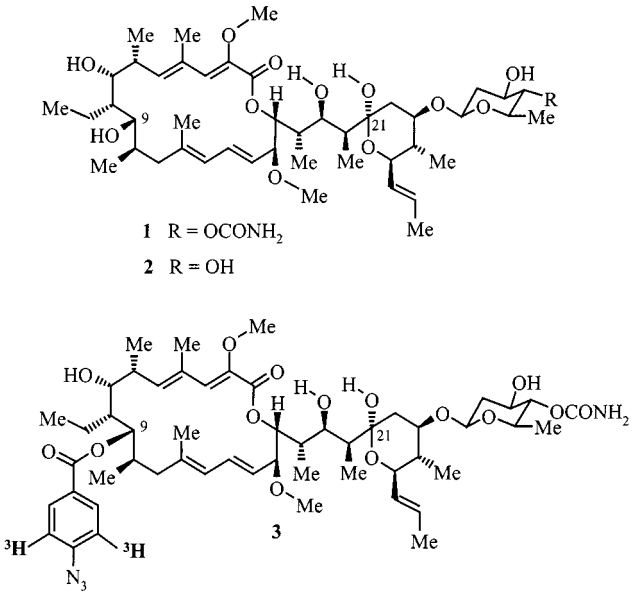

Figure 1. Structure of concanamycins

On the basis of these results we introduced tracer groups (fluorescent or radioactive) and nitrene-generating azido or carbene-generating diazirine groups to achieve the covalent binding of concanamycin derivatives to their target.[13,15]

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The first concanamycin derivative for photoaffinity labelling was 9-*O*-([3,5-^{3}H]-4-azidobenzoyl)-21-deoxyconcanamycin A (**3**), with a specific radioactivity of 10 mCi/mmol.[13] With this derivative, labelling of the Kdp-ATPase (P-type ATPase) was possible, but experiments with V-type ATPases were unsuccessful, probably due to the relatively low specific radioactivity and the low traceability of tritium.[16]

In this paper we present the synthesis of a photoreactive concanamycin derivative that exhibits an inhibitory activity against V- and P-type ATPases and possesses a high specific radioactivity due to the presence of ^{125}I as a tracer element. The covalent binding to the enzymes was achieved through the introduction of a diazirine into the plecomacrolide.

Synthetic Strategy

The concanamycins were originally isolated from *Streptomyces diastatochromogenes*.[17] As described previously,[2,14] we reisolated them from the mycelium extract of a new soil isolate *Streptomyces* sp. (Gö 22/15) in the course of our chemical screening program. Concanamycin A (**1**) was the main component synthesised by this strain,[18] and so we used it as the starting point for derivatisation.

Because of their hemiacetal groups at C(21), concanamycin A (**1**) and, especially, its aglycone concanolide A are rather sensitive to acids and bases. The conditions for any derivatisation are thus limited, as is the half-time during biological tests and photoaffinity labelling experiments. We decided to enhance the stability of the hemiacetal not by acetalisation, which is easily possible, but by production of a deoxy derivative, turning the hemiacetal into a cyclic ether. The method for this reduction was first reported by Horne and Jordan.[20] Furthermore, we decided to liberate the aglycone of the plecomacrolide by hydrolysis, because we had seen previously that the loss of the sugar moiety does not affect the inhibitory activity.[4] We thus focused our interest on 21-deoxyconcanolide A (**6**), which exhibited an inhibitory potential on ATPases almost identical to that of **1**.[15]

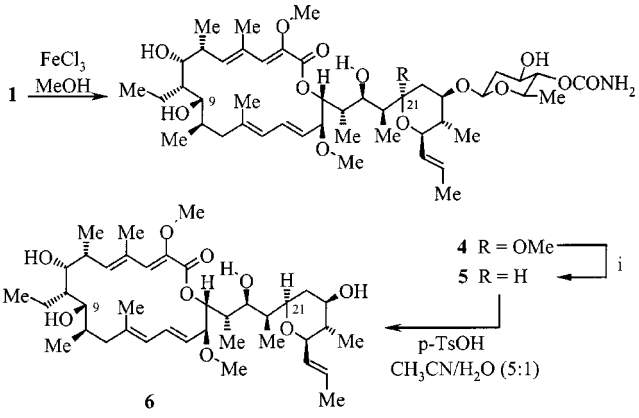
As photolabel, the very effective carbene-generating diazirine group was chosen as part of a *p*-(trifluoroethyldiazirinyl)benzoyl residue,[19] which could easily be introduced by esterification. Because of its high traceability, ^{125}I was selected as a radioactive tracer and introduced by means of a simple nucleophilic substitution of a suitable leaving group with ^{125}I$^-$. For reasons of expense and safety, this reaction was planned as the last step of the whole synthesis. As a consequence, the esterification with *p*-(trifluoroethyldiazirinyl)benzoic acid had to be performed one step earlier.

For 21-deoxyconcanolide A (**6**), it had previously been shown that esterification of the hydroxy groups can be performed regioselectively, starting with 23-OH and followed by 9-OH.[12,13] As 7-OH and 19-OH remained unaffected, it appeared that they were hindered by intramolecular conformational constraints and by a rigid hydrogen bonding system, respectively. A selective esterification of 9-OH was only possible with prior protection of 23-OH. Nosylate protection of the latter appeared appropriate, because nosylate's leaving group properties would simultaneously be advantageous for substitution with Na^{125}I in the last step. Esterification with *p*-(trifluoroethyldiazirinyl)benzoic acid at 9-OH before introduction of the radioactive tracer appeared feasible.

Results

21-Deoxyconcanolide A (6)

The transformation of concanamycin A (**1**) into the more stable 21-deoxyconcanolide A (**6**) was performed in three steps (Scheme 1). The reversible 21-*O*-methylation with 10^{-3} M HCl in MeOH has already been used for the separation of concanamycins A (**1**) and B.[17] When we followed the reported reaction conditions, we observed partial degradation of **1**. The quantitative transformation of **1** into its 21-*O*-methyl derivative **4** could, however, be achieved in methanol with catalytic amounts of Lewis acids, such as 0.1 equivalent of $FeCl_3$, within a few minutes. The ^{1}H NMR spectroscopic data of the product showed the introduction of a methoxy group (δ_H = 3.02). As the NMR chemical shifts and coupling constants of **1** and **4** were almost identical, it could be deduced that the acetalisation had proceeded regio- and stereoselectively with retention of the configuration at C(21).



Scheme 1. Synthesis of 21-deoxyconcanolide A (**6**) (Reagents: i, $NaBH_3CN$, HCl, EtOH)

For the reduction of the acetal into a cyclic ether we chose the mild approach described by Horne and Jordan with $NaBH_3CN$/HCl(g)/MeOH.[20] Modified conditions, with aqueous HCl and EtOH as solvent, enabled 21-*O*-methylconcanamycin A (**4**) to be transformed into 21-deoxyconcanamycin A (**5**) by a quick route with a yield of 46%. This reaction, which probably involves the formation of an oxonium ion as an intermediate, proceeds highly stereospecifically. Direct assignment of the absolute stereochemistry at C(21) of **5** from its ^{1}H NMR coupling constants was impossible because of the overlapping proton signals. For this reason we prepared the 9,3′-di-*O*-acetyl-21-deoxyconcanamycin A (**7**) by treatment of **5** with acetic anhydride/pyridine at room temperature. Extensive NMR studies with different solvents ($CDCl_3$ and C_6D_6) and 2D

methods [^{1}H,^{1}H COSY, Delay-COSY and ^1J(C,H)-COSY] allowed all proton signals and coupling constants to be assigned. The magnitude of the diagnostically important $J_{21,22ax}$ (11.5 Hz) clearly indicates the axial orientation of 21-H (Table 1).

Table 1. Selected ^{1}H NMR (500 MHz) data of the diacetates **7** and **8** (J in Hz)

H atom	**7**[a]	**7**[b]	**8**[a]	Coupling pattern
3	6.39	6.68	6.39	s
9	4.84	5.04	4.83	d (11.0)
16	3.83	3.88	3.80	dd (9.0, 9.0)
17	5.17	5.65	5.16	dd (9.0, 1.5)
19	3.60	4.12	3.59	ddd (10.0, 5.0, 1.5)
21	3.42	3.71	3.56	ddd (11.0, 9.0, 0.5)
22_{eq}	2.10	2.24	2.15	dd (12.0, 5.0)
23	3.49	3.57	4.64	ddd (10.0, 10.0, 5.0)
25	3.29	3.34	3.40	dd (10.0, 7.0)
26	5.34	5.37	5.43	ddq (15.0, 7.0, 1.0)
27	5.58	5.44	5.60	ddq (15.0, 6.0, 1.0)
28	1.62	1.38	1.62	dd (6.0, 1.0)
1′	4.65	4.53	-	dd (10.0, 2.0)
$2'_{eq}$	2.22	2.30	-	ddd (12.0, 5.0, 2.0)
3′	4.98	5.24	-	ddd (11.0, 9.0, 5.0)
4′	4.60	4.93	-	dd (9.0, 9.0)
5′	3.42	3.29	-	dd (9.0, 6.0)

[a] In $CDCl_3$; [b] In C_6D_6.

Because of the lack of hemiacetal activation, deglycosylation of **5** turned out to be more difficult than that described for **1**:[14] the use of large amounts of *p*-toluenesulfonic acid in MeOH/1 M HCl and a long reaction time gave the desired 21-deoxyconcanolide A (**6**) in moderate yield. After modification of the reaction conditions, the yield could be increased to 34% by use of AcCN/H_2O (5:1) at 38 °C for 12 hours. In contrast to the deglycosylation of **1**, no 23-*O*-methylation was observed in the presence of methanol.[14] This showed that the reaction mechanism was different from that of the methanolysis of the hemiacetal-containing concanamycin A (**1**). Finally, to obtain better resolved NMR spectra (Table 1), **6** was transformed into the corresponding 9,23-di-*O*-acetyl-21-deoxyconcanolide A (**8**; Figure 2). The ^{1}H NMR spectra of **6** and **8** indicated that the aglycone had been obtained, since the characteristic deoxyrhamnose signals seen in **5** could not be observed. Detailed analysis of the coupling constants of **8** established that the deglycosylation proceeded with retention of configuration. It should be mentioned that all deoxygenated concanamycins, especially **6**, have much better stability against acids and bases.

23-Iodo(^{125}I)-9-*O*-[*p*-(trifluoroethyldiazirinyl)benzoyl]-21,23-dideoxyconcanolide A (11b)

As described above, the first step towards the target molecule **11b** was the protection of 23-OH by nosylation. Compound **6** was treated with *p*-nitrobenzenesulfonyl chloride at 0 °C in the presence of DMAP/NEt_3 to yield **9** (89%; Scheme 2). The ESI mass spectrum confirmed the molecular mass, with a peak at m/z = 884 $[M + Na]^+$. In

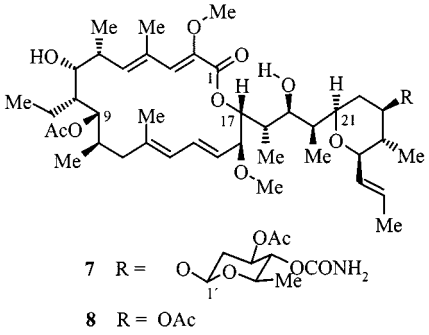
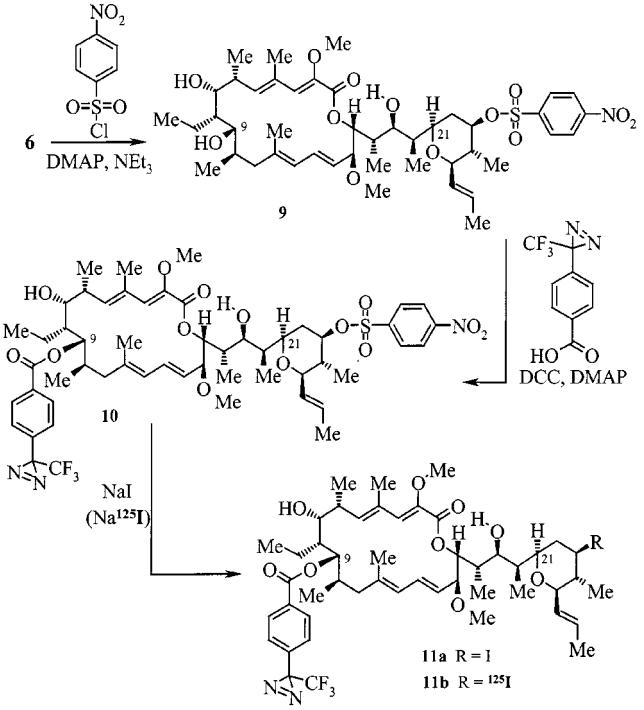


Figure 2. Structure of diacetates generated from **5** and **6**

the ^{1}H NMR spectrum of **9** the new aromatic protons were clearly visible at δ_H = 8.12 and 8.40 and the 23-H signal was shifted downfield from its position in **6** ($\Delta\delta_H$ = 1.66).



Scheme 2. Synthesis of **11a/11b**

For the subsequent esterification of **9** at 9-OH with *p*-(trifluoroethyldiazirinyl)benzoic acid numerous methods were available. On the basis of our previous experience with several concanamycin derivatives we used DCC for the reaction.[21] Treatment of **9** with DCC/DMAP and *p*-(trifluoroethyldiazirinyl)benzoic acid in dichloromethane resulted in the formation of **10** within 18 h (yield: 46%). The esterification of only one hydroxy group was confirmed by the ESI mass spectrum, with a peak at m/z = 1096 $[M + Na]^+$. The ^{1}H NMR spectrum revealed additional signals due to the new aromatic ring (δ_H = 7.30, 8.08) and a downfield shift of 9-H to δ_H = 5.08 ($\Delta\delta_H$ = 1.86 compared with **9**), which indicated the esterification of 9-OH. The ^{13}C NMR signals, especially the quaternary carbon atom at δ_C = 121.2 for the diazirine residue, were clearly attributable to structure **10**.

Table 3. Selected NMR spectroscopic data of **11a** and its 23-epi diastereomer

Atom	**11a**		23-*epi*-**11a**[13]	
	^{1}H NMR[a]	^{13}C NMR[a]	^{1}H NMR[b]	^{13}C NMR[ba]
21	3.38 (m)	80.5	3.97 (ddd, 10.0, 7.0, 1.5)	75.1
22_{ax}	2.05−2.14 (m)	45.4	1.85 (m)	40.6
22_{eq}	2.57 (ddd, 13.0, 4.5, 1.5)		2.30 (m)	
23	4.08 (ddd, 12.0, 12.0, 4.5)	38.7	4.74 (ddd, 2.5, 2.5, 3.0)	46.0
24	1.68 (m)	46.3	0.53 (m)	40.0
24-Me	0.96 (d, 6.5)	19.5	0.78 (d, 6.5)	20.8
25	3.40 (m)	84.2	3.66 (m)	80.9
26	5.31 (m)	131.2	5.34 (ddq, 15.0, 7.5, 2.0)	130.3
27	5.56 (dq, 15.0, 6.5)	129.0	5.61 (dq, 15.0, 6.5)	129.7
28	1.59 (m)	17.8	1.61 (dd, 6.5, 1.5)	18.1

[a] 500 MHz, CD_2Cl_2, *J* in Hz; [b] 500 MHz, $CDCl_3$, *J* in Hz.

In the last step, the nucleophilic substitution of the nosylate moiety with sodium iodide was achieved in good yields (92%) by stirring **10** in the presence of seven equivalents of NaI in DMF at 40 °C for 3 h.[22] The ^{13}C NMR spectrum of the product showed the signal of C(23) at δ_C = 38.7, with an expected upfield shift from that in **10** (δ_C = 87.7). From the coupling constants in the ^{1}H NMR spectrum of **11a**, in conjunction with its ^{1}H,^{1}H-COSY spectrum, this reaction proceeds with retention of the stereochemistry of C(23), indicating either an S_N1 mechanism or a repeated S_N2 process with neighbouring group participation (e.g., 19-OH). The vicinal coupling constant $J_{23,24}$ = 12 Hz (**10**: $J_{23,24}$ = 11 Hz) clearly indicates the axial orientation of 23-H, which represents the configuration of lower energy.[23]

An impurity (about 10%) could be detected in the ^{1}H NMR spectrum of **11a**. In particular, the signal of 23-H had shifted from δ_H = 4.08 to 4.74 and its coupling constants had changed, indicating the equatorial orientation of 23-H in the by-product (Table 2).[13] The data for **11a** and its 23-*epi*-iodo diastereomer (Figure 3) are in agreement with those of iodocyclohexane derivatives.[24]

The inhibitory efficacy of **11a** against V- and P-type ATPases is not as high as that of **6** (Table 3), but it still is an effective inhibitor and therefore suitable for labelling experiments. Furthermore, it was necessary that the required radioactive **11b** should exhibit a high specific radioactivity. To achieve this, we treated **10** with only 2.5 equivalent NaI in addition to 10 mCi Na^{125}I. Because of the lower concentration of NaI, the reaction time was increased. After 48 h we obtained 23-iodo(^{125}I)-9-*O*-[*p*-(trifluoroethyldiazirinyl)benzoyl]-21,23-dideoxyconcanolide A (**11b**) in a yield of 50% and with a specific radioactivity of 438.7 mCi/mmol.

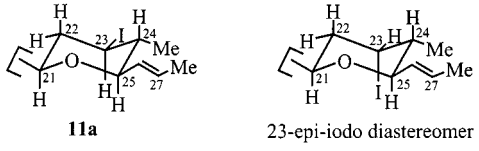


Figure 3. Partial structure of **11a** and its epimer

Discussion

The photoreactive derivative of concanamycin **11a** was tested as an inhibitor against *Manduca sexta* V-type ATPase and *E. coli* Kdp-ATPase.[15,25] As shown in Table 3, compound **11a** inhibited the V-ATPase half maximally at a concentration of 15−20 μM, equivalent to an I_{50} value of approximately 0.8 μmol per mg of protein and the Kdp-ATPase with a K_i value of 40 μM. Unfortunately, **11a** is not as potent an inhibitor as concanamycin A (**1**) and 21-deoxyconcanamycin A (**6**), although its remaining activity for the V-type ATPase is high enough for subsequent binding studies.

Labelling experiments with the purified Kdp-ATPase showed reactions between **11b** and subunits KdpA and KdpB, as already observed with 9-*O*-[(3,5-^{3}H)-4-azidobenzoyl]-21-deoxyconcanamycin A (**3**).[16] However, neither concanamycin A (**1**) nor bafilomycin B_1 quenched this covalent modification.[15] Thus, the specificity of this covalent modification remains ambiguous.

UV irradiation of the purified *M. sexta* V-ATPase in the presence of **11b** resulted in a specific labelling of the V-ATPase subunit c.[25] The specificity of this reaction is corroborated by the fact that the covalent modification can be

Table 3. Inhibitory activity of **1**, **6** and **11a** against purified *E. coli* Kdp-ATPase and the V-type ATPases of *N. crassa* and *M. sexta*

	Kdp-ATPase K_i (μM)/I_{50} (μmol/mg)	V-ATPase (*N. crassa*) K_i (nM)/I_{50} (μmol/mg)	V-ATPase (*M. sexta*) K_i (μM)/I_{50} (μmol/mg)
1	1.2/0.11	$1.2/0.12\cdot10^{-3}$	$0.01/0.5\cdot10^{-3}$
6	15/1.36	$0.9/0.09\cdot10^{-3}$	not tested
11a	40/4.1	not tested	15−20/0.8

quenched by an excess of concanamycin A (**1**) or bafilomycin B_1. This labelling experiment provided the first direct evidence that subunit c, which forms the major part of the proton-translocating V_0 complex of V-type ATPases, contains the binding site for plecomacrolides.

In summary, **11b** represents an important derivative for the determination of the binding site of concanamycin-type compounds in V-type ATPases. Further experiments with V-type ATPases to define the site of covalent modification more precisely are in progress. Compound **11b** was not suitable for the identification of the plecomacrolide binding site within the Kdp-ATPase. A specific labelling of this ATPase should probably be achievable with a derivative of a higher specific radioactivity and especially with a better specific inhibitory potential.

To modify multifunctional antibiotics for binding studies with target enzymes, detailed knowledge about the chemical behaviour of the parent compounds should be available in order that appropriate derivatives may be synthesised. In the case of the concanamycins there seems to be a relatively high tolerance to modification without loss of the specific ATPase inhibitor activity. In particular, 9-OH and 23-OH of **6** can be modified widely, and these are fortunately the most reactive functional groups of the molecule, while the others are so deactivated that they seem to be protected by internal conformational constraints. Thus, the plecomacrolides are good examples of the value and potential of semisynthetic studies of multifunctional compounds.

Experimental Section

General Remarks: Melting points: Reichert hot stage microscope (not corrected); IR spectra: Perkin−Elmer 298 spectrometer; UV spectra: Varian Cary 3E spectrophotometer. Optical rotation: Perkin−Elmer 241; CD spectra: JASCO J 500 A spectrometer; FAB-MS: Finnigan MAT 8230 with nitrobenzyl alcohol as matrix; ESI-MS: Finnigan LQC; ^{1}H and ^{13}C NMR spectra: Varian VXR 500 (500 MHz). Chemical shifts are expressed in δ values (ppm) using the solvent as internal reference. The multiplicities of the ^{13}C NMR values were assigned by attached proton test (APT). TLC: silica gel plates (60 F_{245} silica gel on aluminium foil, Merck), RP-8 (F_{254}S, Merck). LC: silica gel ICN Sili Tech 32−63 (0.032−0.063 mm, ICN Biomedicals GmbH), Sephadex LH-20 (Pharmacia), RP-8 (Merck, Lobar, size B). Staining reagent: vanillin/sulfuric acid: 1 g of vanillin in 100 mL of sulfuric acid. Concanamycin: Isolated from the mycelium of *Streptomyces* strain Gö 22/15 and purified as described elsewhere.[18] Usual workup procedure: threefold extraction of the reaction mixture with the indicated solvent, washing with water, drying with Na_2SO_4 and removal of the solvent in vacuo.

21-*O*-Methylconcanamycin A (4): A mixture of concanamycin A (**1**) (800 mg, 0.92 mmol) in MeOH (20 mL) was treated with $FeCl_3$ (15 mg, 0.09 mmol) and stirred for 15 min. After addition of phosphate buffer (pH = 7, 20 mL), MeOH was removed in vacuo and the aqueous residue was extracted with ethyl acetate. The usual workup procedure was followed by a short filtration through silica gel (acetone/cyclohexane, 2:3) to yield **4** (780 mg, 96%) as a white foam. M.p. 128 °C (dec.); IR (KBr): $\tilde{\nu}$ = 3455 cm^{-1}, 2966, 1709, 1680 sh, 1621 w, 1454, 1380, 1250, 1102; UV (MeOH): λ_{max} (log ε) = 244 (4.55), 282 (4.41); $[\alpha]_D^{20}$ = +46 (*c* = 0.4 in MeOH); CD (MeOH): $\lambda_{extr.}$ ($[\Theta]^{22}$) = 211 nm (sh) (7700), 242 (84800), 267 (−49200); ^{1}H NMR (500 MHz, CD_2Cl_2/CD_3OD, 2:1): δ = 0.85 (d, *J* = 7.0 Hz, 3H 24-CH_3), 0.85 (m, 3 H, 8-Et-CH_3), 0.86 (d, *J* = 7.0 Hz, 3 H, 18-CH_3), 0.98 (d, *J* = 7.0 Hz, 3 H, 20-CH_3), 1.02 (d, *J* = 7.0 Hz, 3 H, 6-CH_3), 1.04 (m, 3 H, 10-CH_3), 1.22 (m, 2 H, 8-Et-CH_2), 1.24 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 1.26 (m, 1 H, 24-H), 1.42 (m, 1 H, 8-H), 1.47 (m, 1 H, 22-H_{ax}), 1.62 (m, 1 H, 2′-H_{ax}), 1.70 (dd, *J* = 6.5, 1.5 Hz, 3 H, 28-H), 1.81 (s, 3 H, 12-CH_3), 1.89 (m, 2 H, 11-H_2), 1.97 (m, 1 H, 18-H), 1.99 (s, 3 H, 4-CH_3), 2.05 (m, 1 H, 20-H), 2.22 (ddd, *J* = 12.0, 5.0, 2.0 Hz, 1 H, 2′-H_{eq}), 2.29 (m, 1 H, 10-H), 2.33 (dd, *J* = 13.0, 5.0 Hz, 1 H, 22-H_{eq}), 2.75 (m, 1 H, 6-H), 3.02 (s, 3 H, 21-OCH_3), 3.09 (m, 1 H, 9-H), 3.23 (s, 3 H, 16-OCH_3), 3.35−3.44 (m, 2 H, 19-H and 5′-H), 3.48 (dd, *J* = 10.0, 8.0 Hz, 1 H, 25-H), 3.58 (m, 1 H, 7-H), 3.63 (m, 1 H, 23-H), 3.60 (m, 1 H, 3′-H), 3.64 (s, 3 H, 2-OCH_3), 3.80 (dd, *J* = 9.0, 9.0 Hz, 1 H, 16-H), 4.28 (dd, *J* = 9.0, 9.0 Hz, 1 H, 4′-H), 4.56 (dd, *J* = 10.0, 1.0 Hz, 1 H, 1′-H), 5.07 (dd, *J* = 9.0, 0.5 Hz, 1 H, 17-H), 5.23 (dd, *J* = 15.0, 9.0 Hz, 1 H, 15-H), 5.40 (ddq, *J* = 15.0, 8.0, 1.5 Hz, 1 H, 26-H), 5.66 (dq, *J* = 15.0, 6.5 Hz, 1 H, 27-H), 5.69 (m, 1 H, 5-H), 5.78 (d, *J* = 11.0 Hz, 1 H, 13-H), 6.45 (s, 1 H, 3-H), 6.50 (dd, *J* = 15.0, 11.0 Hz, 1 H, 14-H); ^{13}C NMR (125 MHz, CD_2Cl_2/CD_3OD, 2:1, −20 °C): δ = 7.2 (q, 20-CH_3), 10.0 (q, 18-CH_3), 11.6 (q, 8-Et-CH_3), 13.2 (q, 24-CH_3), 13.9 (q, 4-CH_3), 16.0 (q, 12-CH_3), 16.7 (q, 6-CH_3), 17.4 (q, C-6′), 17.8 (q, C-28), 21.7 (q, 10-CH_3), 22.5 (t, 8-Et-CH_2), 34.7 (d, C-6), 34.9 (d, C-10), 35.7 (t, C-22), 37.8 (d, C-20), 38.1 (d, C-18), 39.6 (t, C-2′), 40.3 (d, C-24), 43.6 (d, C-8), 45.3 (t, C-11), 46.4 (q, 21-OCH_3), 55.7 (q, 16-OCH_3), 60.0 (q, 2-OCH_3), 69.0 (d, C-3′) 69.5 (d, C-19), 69.8 (d, C-5′), 73.2 (d, C-23), 75.2 (d, C-7), 75.7 (d, C-17), 76.9 (d, C-25), 78.6 (d, C-4′), 79.4 (d, C-9), 82.3 (d, C-16), 95.9 (d, C-1′), 103.6 (s, C-21), 122.8 (d, C-13), 126.5 (d, C-15), 130.1 (d, C-27), 130.3 (d, C-26), 131.5 (s, C-4), 132.2 (d, C-3), 133.8 (d, C-14), 141.4 (d, C-5), 141.5 (s, C-2), 142.7 (s, C-12), 157.9 (s, $CONH_2$), 166.6 (s, C-1); FAB MS: *m*/*z* = 879 [M − H] (neg.); 903 [M + Na] (pos.); $C_{47}H_{77}NO_{14}$ (880.12).

21-Deoxyconcanamycin A (5): A solution of **4** (710 mg, 0.81 mmol) and $NaBH_3CN$ (361 mg, 4.86 mmol, 6 equiv.) in EtOH (70 mL) was treated with HCl (0.5 M, 7 mL) and stirred at room temp. After 4 h, phosphate buffer (60 mL) was added, EtOH was removed in vacuo and the resulting aqueous solution was extracted with CH_2Cl_2. The usual workup procedure followed by CC (RP-8, MeOH/H_2O, 8:2) gave **5** (302 mg, 46%) as a white, amorphous powder. M.p. 94 °C (dec.); IR (KBr): $\tilde{\nu}$ = 3443 cm^{-1}, 2927, 1712, 1620 w, 1455 w, 1381, 1252, 1102; UV (MeOH): λ_{max} (log ε) = 244 (4.46), 282 (4.11); $[\alpha]_D^{20}$ = −46 (*c* = 1 in MeOH); CD (MeOH): $\lambda_{extr.}$ ($[\Theta]^{22}$) = 210 nm (sh) (7500), 242 (69600), 267 (−43100); ^{1}H NMR (500 MHz, CD_2Cl_2/CD_3OD, 2:1): δ = 0.81 (d, *J* = 7.0 Hz, 3 H, 18-CH_3), 0.83 (d, *J* = 7.0 Hz, 3H 24-CH_3), 0.84 (t, *J* = 7.0 Hz, 3 H, 8-Et-CH_3), 0.85 (d, *J* = 7.0 Hz, 3 H, 20-CH_3), 0.96 (m, 3 H, 6-CH_3), 1.07 (d, *J* = 7.0 Hz, 3 H, 10-CH_3), 1.08 (m, 1 H, 22-H_{ax}), 1.09 (m, 2 H, 8-Et-CH_2), 1.19 (m, 1 H, 24-H), 1.21 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 1.41 (m, 1 H, 8-H), 1.56 (m, 1 H, 2′-H_{ax}), 1.61 (dd, *J* = 6.0, 1.5 Hz, 3 H, 28-H), 1.64 (m, 1 H, 20-H), 1.83 (m, 2 H, 11-H_2), 1.96 (s, 3 H, 12-CH_3), 1.99 (m, 1 H, 18-H), 2.00 (s, 3 H, 4-CH_3), 2.09 (m, 1 H, 2′-H_{eq}), 2.13 (m, 1 H, 22-H_{eq}), 2.26 (m, 1 H, 10-H), 2.71 (m, 1 H, 6-H), 3.20 (m, 1 H, 9-H), 3.23 (s, 3 H, 16-OCH_3), 3.30 (dd, *J* = 10.0, 8.0 Hz, 1 H, 25-H), 3.34 (m, 1 H, 5′-H), 3.38 (m, 1 H, 21-H), 3.48 (ddd, *J* = 11.0, 11.0, 4.5 Hz, 1 H, 23-H), 3.54 (s, 3 H, 2-OCH_3), 3. 57 (dd, *J* = 10.0, 1.5 Hz, 1 H, 19-H), 3.67 (m, 1 H, 3′-H), 3.70 (m, 1 H, 7-H), 3.80 (dd, *J* = 9.0, 9.0 Hz, 1 H, 16-H), 4.20 (dd, *J* = 9.0, 9.0 Hz, 1 H, 4′-H), 4.59 (dd, *J* = 10.0, 1.5 Hz, 1 H, 1′-H), 5.11 (m, 1 H, 17-H), 5.19 (dd, *J* =

15.0, 9.0 Hz, 1 H, 15-H), 5.32 (ddq, *J* = 15.0, 8.0, 1.5 Hz, 1 H, 26-H), 5.57 (dq, *J* = 15.0, 6.0 Hz, 1 H, 27-H), 5.69 (m, 1 H, 5-H), 5.80 (d, *J* = 11.0 Hz, 1 H, 13-H), 6.39 (s, 1 H, 3-H), 6.55 (dd, *J* = 15.0, 11.0 Hz, 1 H, 14-H); ^{13}C NMR (125 MHz, CD_2Cl_2/CD_3OD, 2:1, −20 °C): δ = 7.9 (q, 20-CH_3), 9.3 (q, 18-CH_3), 11.7 (q, 8-Et-CH_3), 13.2 (q, 24-CH_3), 14.1 (q, 4-CH_3), 16.1 (q, 12-CH_3), 16.8 (q, 6-CH_3), 17.3 (q, C-6′), 17.8 (q, C-28), 21.7 (q, 10-CH_3), 22.6 (t, 8-Et-CH_2), 34.8 (d, C-6), 34.8 (t, C-22), 35.6 (d, C-10), 37.2 (d, C-18), 39.1 (d, C-20), 39.6 (t, C-2′), 41.2 (d, C-24), 43.5 (d, C-8), 45.2 (t, C-11), 55.7 (q, 16-OCH_3), 59.2 (q, 2-OCH_3), 69.1 (d, C-3′) 69.2 (d, C-19), 69.9 (d, C-5′), 73.3 (d, C-7), 75.8 (d, C-17), 76.2 (d, C-21), 78.1 (d, C-23), 78.5 (d, C-4′), 79.4 (d, C-9), 82.1 (d, C-16), 82.9 (d, C-25), 95.6 (d, C-1′), 122.9 (d, C-13), 126.7 (d, C-15), 128.7 (d, C-27), 130.6 (d, C-26), 131.6 (s, C-4), 132.1 (d, C-3), 133.8 (d, C-14), 140.9 (d, C-5), 141.7 (s, C-2), 142.7 (s, C-12), 158.1 (s, $CONH_2$), 166.2 (s, C-1); FAB MS: *m*/*z* = 849 [M − H] (neg.); 873 [M + Na] (pos.); $C_{46}H_{75}NO_{13}$ (850.09).

3′,9-Di-*O*-acetyl-21-deoxyconcanamycin A (7): A solution of **5** (36 mg, 0.042 mmol) in pyridine (0.6 mL) was treated with acetic anhydride (0.5 mL) and stirred for 3 h at room temp. The mixture was poured into ice-water and extracted with $CHCl_3$. After the usual workup procedure, traces of acid were removed in vacuo by addition of toluene. Purification by CC (EtOAc/*n*-hexane, 3:2) gave **7** (40 mg, 77%). M.p. 133 °C (dec.); IR (KBr): $\tilde{\nu}$ = 3500 cm^{-1}, 2970, 1740, 1720sh, 1690sh, 1620 w, 1450, 1380, 1240, 1110; UV (MeOH): λ_{max} (log ε) = 245 (4.56), 284 (4.20); $[\alpha]_D^{20}$ = −45 (*c* = 0.5 in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 2; ^{1}H NMR (500 MHz, [D_6]benzene) see Table 2 and δ = 0.93 (t, *J* = 7.0 Hz, 3 H, 8-Et-CH_3), 0.95 (d, *J* = 7.0 Hz, 3 H, 6-CH_3, 18-CH_3), 0.95 (m, 1 H, 8-Et-CH_2), 1.08 (d, *J* = 7.0 Hz, 3 H, 20-CH_3), 1.10 (m, 1 H, 8-Et-CH_2), 1.11 (d, *J* = 7.0 Hz, 3 H, 24-CH_3), 1.33 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 1.35 (ddd, *J* = 12.0, 11.0, 10.0 Hz, 1 H, 22-H_{ax}), 1.55 (ddq, *J* = 10.0, 10.0, 7.0 Hz, 1 H, 24-H), 1.65 (m, 1 H, 8-H), 1.78 (s, 6 H, 12-CH_3, H_3C-COO), 1.89 (ddd, *J* = 12.0, 11.0, 10.0 Hz, 1 H, 2′-H_{ax}), 1.93 (dq, *J* = 9.0, 7.0 Hz, 1 H, 20-H), 1.97 (m, 2 H, 11-H_2), 2.07 (s, 6 H, 4-CH_3, H_3C-COO), 2.55 (m, 2 H, 6-H, 18-H), 2.71 (m, 1 H, 10-H), 3.10 (s, 3 H, 16-OCH_3), 3.64 (s, 3 H, 2-OCH_3), 5.18 (dd, *J* = 15.0, 9.0 Hz, 1 H, 15-H), 5.75 (d, *J* = 10.0 Hz, 1 H, 13-H), 5.82 (d, *J* = 10.0 Hz, 1 H, 5-H), 6.50 (dd, *J* = 15.0, 10.0 Hz, 1 H, 14-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.2 (q, 20-CH_3), 9.6 (q, 18-CH_3), 11.9 (q, 8-Et-CH_3), 14.1 (q, 4-CH_3), 16.2 (q, 12-CH_3), 16.9 (q, 6-CH_3), 17.5 (q, C-6′),17.6 (q, C-28), 21.5 (q, 10-CH_3), 21.5 (t, 8-Et-CH_2), 33.9 (d, C-6), 34.8 (t, C-22), 35.0 (d, C-10), 37.0 (t, C-2′), 37.3 (d, C-18), 39.5 (d, C-20), 41.4 (d, C-24), 43.9 (d, C-8), 45.3 (t, C-11), 55.7 (q, 16-OCH_3), 59.2 (q, 2-OCH_3), 69.6 (d, C-19), 71.0 (d, C-3′), 70.1 (d, C-5′),75.0 (d, C-7), 75.3 (d, C-4′), 76.1 (d, C-17), 76.8 (d, C-21), 78.8 (d, C-23), 79.7 (d, C-9), 82.0 (d, C-16), 82.9 (d, C-25), 95.5 (d, C-1′),123.8 (d, C-13), 128.0 (d, C-15), 128.2 (d, C-27), 130.8 (d, C-26), 130.0 (d, C-3), 132.4 (s, C-4), 133.0 (d, C-14), 138.6 (d, C-5), 141.2 (s, C-2), 142.4 (s, C-12), 155.9 (s, $CONH_2$), 165.9 (s, C-1); ESI MS: *m*/*z* = 957 [M + Na] (pos); $C_{50}H_{79}NO_{15}$ (934.11).

21-Deoxyconcanolide A (6): A mixture of **5** (302 mg, 0.37 mmol) and *p*-toluenesulfonic acid (164 mg, 0.85 mmol, 2.3 equiv.) in AcCN/H_2O (30 mL, 5:1) was stirred for 12 h at 38 °C. After cooling to 0 °C and addition of 30 mL sat. $NaHCO_3$ solution, the organic solvent was removed in vacuo and the resulting aqueous solution was extracted with CH_2Cl_2. The usual workup procedure, followed by CC (EtOAc/cyclohexane, 2:3), gave **6** (82 mg, 34%) as a white, amorphous powder and 90 mg (30%) starting material. M.p. 97 °C (dec.); IR (KBr): $\tilde{\nu}$ = 3456 cm^{-1}, 2965, 1696, 1650 w, 1623 w, 1454, 1361, 1250, 1104; UV (MeOH): λ_{max} (log ε) = 243 (4.40), 281 (4.06); $[\alpha]_D^{20}$ = −23 (*c* = 0.6 in MeOH); CD (MeOH): $\lambda_{extr.}$ ($[\Theta]^{22}$) = 212 nm (sh) (11600), 242 (99500), 267 (−65100); ^{1}H NMR (500 MHz, CD_2Cl_2/CD_3OD, 2:1): δ = 0.81 (d, *J* = 7.0 Hz, 3 H, 18-CH_3), 0.82 (m, 3 H, 8-Et-CH_3), 0.85 (d, *J* = 7.0 Hz, 3 H, 20-CH_3), 0.87 (d, *J* = 6.5 Hz, 3H 24-CH_3), 1.02 (m, 3 H, 6-CH_3), 1.04 (d, *J* = 7.0 Hz, 3 H, 10-CH_3), 1.11 (m, 2 H, 8-Et-CH_2), 1.17 (m, 1 H, 24-H), 1.20 (m, 1 H, 22-H_{ax}), 1.46 (m, 1 H, 8-H), 1.61 (dd, *J* = 6.0, 1.5 Hz, 3 H, 28-H), 1.64 (m, 1 H, 20-H), 1.84 (s, 3 H, 12-CH_3), 1.80−1.90 (m, 2 H, 11-H_2), 1.96 (s, 3 H, 4-CH_3), 2.03 (m, 1 H, 22-H_{eq}), 2.04 (m, 1 H, 18-H), 2.26 (m, 1 H, 10-H), 2.71 (m, 1 H, 6-H), 3.20 (m, 1 H, 9-H), 3.23 (s, 3 H, 16-OCH_3), 3.27 (m, 1 H, 23-H), 3.31 (m, 1 H, 25-H), 3.43 (dd, *J* = 11.0, 8.0, 1.5 Hz, 1 H, 21-H), 3.54 (s, 3 H, 2-OCH_3), 3. 59 (dd, *J* = 10.0, 1.5 Hz, 1 H, 19-H), 3.61 (m, 1 H, 7-H), 3.80 (dd, *J* = 9.0, 9.0 Hz, 1 H, 16-H), 5.14 (br. d, *J* = 6.0 Hz, 1 H, 17-H), 5.19 (dd, *J* = 15.0, 9.0 Hz, 1 H, 15-H), 5.32 (ddq, *J* = 15.0, 8.0, 1.5 Hz, 1 H, 26-H), 5.57 (dq, *J* = 15.0, 6.0 Hz, 1 H, 27-H), 5.69 (d, *J* = 10.0 Hz, 1 H, 5-H), 5.80 (d, *J* = 11.0 Hz, 1 H, 13-H), 6.39 (s, 1 H, 3-H), 6.55 (dd, *J* = 15.0, 11.0 Hz, 1 H, 14-H); ^{13}C NMR (125 MHz, CD_2Cl_2/CD_3OD 2:1, −20 °C): δ = 7.9 (q, 20-CH_3), 9.3 (q, 18-CH_3), 11.7 (q, 8-Et-CH_3), 13.2 (q, 24-CH_3), 14.1 (q, 4-CH_3), 16.1 (q, 12-CH_3), 16.8 (q, 6-CH_3), 17.8 (q, C-28), 21.7 (q, 10-CH_3), 22.6 (t, 8-Et-CH_2), 34.9 (d, C-6), 35.6 (d, C-10), 37.2 (d, C-18), 38.8 (t, C-22), 39.1 (d, C-20), 43.5 (d, C-24), 43.7 (d, C-8), 45.4 (t, C-11), 55.7 (q, 16-OCH_3), 59.2 (q, 2-OCH_3), 69.2 (d, C-19), 73.3 (d, C-23), 73.3 (d, C-7), 75.8 (d, C-17), 76.5 (d, C-21), 79.4 (d, C-9), 82.1 (d, C-16), 82.9 (d, C-25), 122.9 (d, C-13), 126.7 (d, C-15), 128.7 (d, C-27), 130.6 (d, C-26), 131.1 (d, C-3), 131.6 (s, C-4), 133.8 (d, C-14), 140.9 (d, C-5), 141.7 (s, C-2), 142.7 (s, C-12), 166.2 (s, C-1); FAB MS: *m*/*z* = 676 [M − H] (neg.); 699 [M + Na] (pos.); $C_{39}H_{64}O_9$ (676.93).

9,23-Di-*O*-acetyl-21-deoxyconcanolide A (8): Compound **6** (12.8 mg, 0.019 mmol) was acetylated as in the acetylation of **5**. CC (EtOAc/*n*-hexane, 1:1) gave **8** (7.4 mg, 77%); UV (MeOH): λ_{max} (log ε) = 245 (4.59), 284 (4.23); ^{1}H NMR (500 MHz, $CDCl_3$) see Table 2 and δ = 0.77 (d, *J* = 7.0 Hz, 3 H, 24-CH_3), 0.80 (d, *J* = 7.0 Hz, 3 H, 18-CH_3), 0.86 (d, *J* = 7.0 Hz, 3 H, 20-CH_3), 0.90 (d, *J* = 7.0 Hz, 3 H, 10-CH_3), 1.08 (d, *J* = 7.0 Hz, 3 H, 6-CH_3), 1.27 (ddd, *J* = 12.0, 11.0, 11.0 Hz, 1 H, 22-H_{ax}), 1.43 (ddd, *J* = 11.0, 10.0, 6.5 Hz, 1 H, 24-H), 1.52 (m, 1 H, 8-H), 1.68 (m, 1 H, 20-H), 1.86 (s, 3 H, 12-CH_3), 1.94 (s, 3 H, 4-CH_3), 1.9−2.0 (m, 2 H, 11-H_2), 2.04 (s, 3 H, H_3C-COO), 2.05 (m, 1 H, 18-H), 2.10 (s, 3 H, H_3C-COO), 2.56 (m, 1 H, 10-H), 2.67 (ddd, *J* = 10.0, 7.0, 2.5 Hz, 1 H, 6-H), 3.23 (s, 3 H, 16-OCH_3), 3.57 (s, 3 H, 2-OCH_3), 3.66 (m, 1 H, 7-H), 5.25 (dd, *J* = 15.0, 9.0 Hz, 1 H, 15-H), 5.55 (d, *J* = 10.0 Hz, 1 H, 5-H), 5.78 (d, *J* = 10.5 Hz, 1 H, 13-H), 6.54 (dd, *J* = 15.0, 10.5 Hz, 1 H, 14-H); ESI MS: *m*/*z* = 784 [M + Na] (pos); $C_{43}H_{68}O_{11}$ (760.96).

23-*O*-(*p*-Nitrobenzylsulfonyl)-21-deoxyconcanolide A (9): DMAP (179.5 mg, 1.5 mmol, 12.5 equiv.), NEt_3 (1.6 mL) and *p*-nitrobenzenesulfonyl chloride (334 mg, 1.5 mmol, 12.5 equiv.) were added to a solution of **6** (82 mg, 0.012 mmol) in CH_2Cl_2 (5 mL). After 2 h stirring at 0 °C, the mixture was poured into ice-water and extracted with CH_2Cl_2. The usual workup procedure and CC (EtOAc/cyclohexane, 2:3) furnished **9** (94 mg, 89%) . M.p. 75 °C (dec.); IR (KBr): $\tilde{\nu}$ = 3446 cm^{-1}, 2968, 1694, 1619 w, 1534, 1454 w, 1351, 1250, 1187, 1102; UV (MeOH): λ_{max} (log ε) = 245 (4.63), 282 (4.29); $[\alpha]_D^{20}$ = −34 (*c* = 0.6 in MeOH); CD (MeOH): $\lambda_{extr.}$ ($[\Theta]^{22}$) = 210 nm (sh) (7600), 242 (80800), 268 (−49200); ^{1}H NMR (500 MHz, CD_2Cl_2/CD_3OD, 2:1): δ = 0.65 (d, *J* = 6.5 Hz, 3H 24-CH_3), 0.76 (d, *J* = 6.0 Hz, 3 H, 20-CH_3), 0.78 (d, *J* = 6.0 Hz, 3 H, 18-CH_3), 0.82 (m, 3 H, 8-Et-CH_3), 0.96 (m, 3 H, 6-CH_3), 1.04 (m, 2 H, 8-Et-CH_2), 1.14 (d, *J* = 7.0 Hz, 3 H, 10-CH_3), 1.45 (m, 1

H, 8-H), 1.49 (m, 1 H, 22-H_{ax}), 1.50 (m, 1 H, 24-H), 1.60 (dd, J = 6.0, 2.0 Hz, 3 H, 28-H), 1.63 (m, 1 H, 20-H), 1.84 (s, 3 H, 12-CH_3), 1.97 (s, 3 H, 4-CH_3), 1.98 (m, 2 H, 11-H_2), 2.03 (m, 1 H, 18-H), 2.16 (ddd, J = 11.0, 5.0, 1.5 Hz, 1 H, 22-H_{eq}), 2.29 (m, 1 H, 10-H), 2.72 (m, 1 H, 6-H), 3.22 (m, 1 H, 9-H), 3.23 (s, 3 H, 16-OCH_3), 3.34 (dd, J = 10.0, 8.0 Hz, 1 H, 25-H), 3.44 (dd, J = 11.0, 8.0, 1.5 Hz, 1 H, 21-H), 3.50 (m, 1 H, 7-H), 3.54 (s, 3 H, 2-OCH_3), 3.59 (m, 1 H, 19-H), 3.82 (dd, J = 9.0, 9.0 Hz, 1 H, 16-H), 4.43 (ddd, J = 11.0, 11.0, 5.0 Hz, 1 H, 23-H), 5.08 (br. d, J = 7.0 Hz, 1 H, 17-H), 5.19 (dd, J = 15.0, 9.0 Hz, 1 H, 15-H), 5.27 (ddq, J = 15.0, 8.0, 2.0 Hz, 1 H, 26-H), 5.57 (dq, J = 15.0, 6.0 Hz, 1 H, 27-H), 5.68 (d, J = 10.0 Hz, 1 H, 5-H), 5.80 (d, J = 11.0 Hz, 1 H, 13-H), 6.38 (s, 1 H, 3-H), 6.55 (dd, J = 15.0, 11.0 Hz, 1 H, 14-H), 8.12 (d, J = 9.0 Hz, 2 H, 2 × 2′H), 8.40 (d, J = 9.0 Hz, 2 H, 2 × 3′H); ^{13}C NMR (125 MHz, CD_2Cl_2/CD_3OD, 2:1, −20 °C): δ = 7.7 (q, 20-CH_3), 9.2 (q, 18-CH_3), 11.6 (q, 8-Et-CH_3), 13.2 (q, 24-CH_3), 14.0 (q, 4-CH_3), 16.0 (q, 12-CH_3), 16.8 (q, 6-CH_3), 17.7 (q, C-28), 21.6 (t, 8-Et-CH_2), 22.5 (q, 10-CH_3), 34.8 (d, C-6), 35.6 (d, C-10), 36.8 (t, C-22), 37.1 (d, C-18), 39.1 (d, C-20), 41.0 (d, C-24), 43.6 (d, C-8), 45.3 (t, C-11), 55.7 (q, 16-OCH_3), 59.1 (q, 2-OCH_3), 73.2 (d, C-19), 87.2 (d, C-23), 69.0 (d, C-7), 75.6 (d, C-17), 75.8 (d, C-21), 79.4 (d, C-9), 82.0 (d, C-16), 82.4 (d, C-25), 122.8 (d, C-13), 124.7 (d, C-3′), 126.6 (d, C-15), 128.2 (d, C-26), 129.3 (d, C-2′), 129.9 (d, C-27), 131.2 (d, C-3), 131.6 (s, C-4), 133.8 (d, C-14), 141.0 (d, C-5), 141.6 (s, C-2), 142.4 (s, C-12), 142.8 (s, C-1′), 150.6 (s, C-4′), 166.3 (s, C-1); ESI MS: m/z = 884 [M + Na] (pos.); $C_{45}H_{67}NO_{13}S$ (862.09).

23-*O*-(*p*-Nitrobenzylsulfonyl)-9-*O*-[*p*-(trifluoroethyldiazirinyl)-benzoyl]-21-deoxyconcanolide A (10): Compound **9** (40 mg, 0.05 mmol) in dry CH_2Cl_2 (3 mL) was treated with DMAP (24.3 mg, 0.2 mmol, 4 equiv.), DCC (41.3 mg, 0.2 mmol, 4 equiv.) and *p*-(trifluoroethyldiazirinyl)benzoic acid (29.7 mg, 0.15 mmol, 3 equiv.). The resulting mixture was stirred for 18 h at room temp. and the organic solvent was removed in vacuo. The crude extract was purified on silica gel (EtOAc/cyclohexane, 1:3) and then on LH-20 (acetone) to yield **10** (17 mg, 46%). M.p. 100 °C (dec.); IR (KBr): $\tilde{\nu}$ = 3442 cm^{-1}, 2970, 1716, 1698, 1628 w, 1609 w, 1535, 1454, 1349, 1275, 1188, 1158, 1106; UV (MeOH): λ_{max} (log ε) = 244 (4.83), 281 (4.47); $[\alpha]_D^{20}$ = +36 (c = 0.2 in MeOH); CD (MeOH): $\lambda_{extr.}$ ($[\Theta]^{22}$) = 210 nm (−2020), 225 (−10300), 246 (120500), 267 (−27400); ^{1}H NMR (500 MHz, CD_2Cl_2): δ = 0.66 (d, J = 6.5 Hz, 3H 24-CH_3), 0.77 (d, J = 7.0 Hz, 3 H, 20-CH_3), 0.78 (d, J = 7.0 Hz, 3 H, 18-CH_3), 0.90 (m, 3 H, 10-CH_3), 0.91 (m, 3 H, 8-Et-CH_3), 1.02 (d, J = 7.0 Hz, 3 H, 6-CH_3), 1.10 (m, 2 H, 8-Et-CH_2), 1.48 (m, 1 H, 22-H_{ax}), 1.48 (m, 1 H, 24-H), 1.60 (dd, J = 7.0, 1.5 Hz, 3 H, 28-H), 1.64 (m, 1 H, 20-H), 1.70 (m, 1 H, 8-H), 1.91 (s, 3 H, 12-CH_3), 1.94 (s, 3 H, 4-CH_3), 2.05 (m, 1 H, 18-H), 2.09−2.14 (m, 2 H, 11-H_2), 2.17 (ddd, J = 12.0, 5.0, 2.0 Hz, 1 H, 22-H_{eq}), 2.67 (m, 1 H, 6-H), 2.87 (m, 1 H, 10-H), 3.23 (s, 3 H, 16-OCH_3), 3.35 (dd, J = 9.0, 8.0 Hz, 1 H, 25-H), 3.45 (dd, J = 11.0, 8.0, 2.0 Hz, 1 H, 21-H), 3.51 (m, 1 H, 19-H), 3.54 (s, 3 H, 2-OCH_3), 3.77 (dd, J = 10.0, 1.5 Hz, 1 H, 7-H), 3.82 (dd, J = 9.0, 9.0 Hz, 1 H, 16-H), 4.44 (ddd, J = 11.0, 11.0, 5.0 Hz, 1 H, 23-H), 5.04 (dd, J = 9.0, 1.5 Hz, 1 H, 17-H), 5.08 (d, J = 11.0 Hz, 1 H, 9-H), 5.22 (dd, J = 15.0, 9.0 Hz, 1 H, 15-H), 5.27 (ddq, J = 15.0, 8.0, 1.5 Hz, 1 H, 26-H), 5.57 (dq, J = 15.0, 7.0 Hz, 1 H, 27-H), 5.64 (d, J = 10.0 Hz, 1 H, 5-H), 5.84 (d, J = 11.0 Hz, 1 H, 13-H), 6.42 (s, 1 H, 3-H), 6.61 (dd, J = 15.0, 11.0 Hz, 1 H, 14-H), 7.30 (d, J = 8.5 Hz, 2 H, 2 × 3′′H), 8.08 (d, J = 8.5 Hz, 2 H, 2 × 2′′H), 8.13 (d, J = 9.0 Hz, 2 H, 2 × 2′H), 8.40 (d, J = 9.0 Hz, 2 H, 2 × 3′H); ^{13}C NMR (125 MHz, CD_2Cl_2): δ = 8.0 (q, 20-CH_3), 9.6 (q, 18-CH_3), 12.1 (q, 8-Et-CH_3), 13.5 (q, 24-CH_3), 14.2 (q, 4-CH_3), 16.1 (q, 12-CH_3), 16.8 (q, 6-CH_3), 17.8 (q, C-28), 21.6 (t, 8-Et-CH_2), 21.5 (q, 10-CH_3), 34.0 (d, C-10), 35.3 (d, C-6), 37.2 (t, C-22), 37.3 (d, C-18), 39.7 (d, C-20), 41.6 (d, C-24), 44.5 (d, C-8), 45.9 (t, C-11), 55.9 (q, 16-OCH_3), 59.4 (q, 2-OCH_3), 69.7 (d, C-19), 87.7 (d, C-23), 75.1 (d, C-7), 76.1 (d, C-17), 76.5 (d, C-21), 81.4 (d, C-9), 82.3 (d, C-16), 82.6 (d, C-25), 121.2 (s, C-CF_3), 123.4 (s, CF_3), 124.2 (d, C-13), 124.8 (d, 2 × C-3′), 126.8 (d, 2 × C-3′′), 128.1 (d, C-15), 129.5 (d, 2 × C-2′),129.7 (d, C-27), 130.1 (d, C-26), 130.2 (d, 2 × C-2′′) 130.5 (d, C-3), 132.1 (s, C-4), 132.6 (s, C-1′′), 133.8 (s, C-4′′), 133.8 (d, C-14), 139.2 (d, C-5), 142.0 (s, C-2), 142.6 (s, C-12), 143.4 (s, C-1′), 151.0 (s, C-4′), 165.7 (s, COO) 166.2 (s, C-1); ESI MS: m/z = 1096 [M + Na] (pos.); $C_{54}H_{70}F_3N_3O_{14}S$ (1074.22).

23-Iodo-9-*O*-[*p*-(trifluoroethyldiazirinyl)benzoyl]-21,23-dideoxyconcanolide A (11a): A solution of **10** (9 mg, 0.01 mmol) and NaI (10 mg, 0.07 mmol, 7 equiv.) in DMF (3 mL) was stirred for 3 h at 40 °C. Phosphate buffer (pH = 7, 4 mL) was added and the mixture was extracted with CH_2Cl_2. After the usual workup procedure, traces of DMF were removed in vacuo by addition of toluene. Purification by CC (EtOAc/cyclohexane, 1:3) gave **11a** (9 mg, 92%). M.p. 97 °C (dec.); IR (KBr): $\tilde{\nu}$ = 3448 cm^{-1}, 2968, 1719, 1697, 1623 w, 1605 (sh) w, 1344, 1274, 1194, 1159, 1108; UV (MeOH): λ_{max} (log ε) = 243 (4.75), 281 (4.28); $[\alpha]_D^{20}$ = +36 (c = 0.2 in MeOH); CD (MeOH): $\lambda_{extr.}$ ($[\Theta]^{22}$) = 213 nm (−6700), 224 (−10000), 246 (84700), 267 (−21400)- ^{1}H NMR (500 MHz, CD_2Cl_2): δ = 0.79 (d, J = 7.0 Hz, 3 H, 18-CH_3), 0.82 (d, J = 7.0 Hz, 3 H, 20-CH_3), 0.83 (m, 3 H, 10-CH_3), 0.89 (m, 3 H, 8-Et-CH_3), 0.96 (d, J = 6.5 Hz, 3H 24-CH_3), 1.00 (d, J = 7.0 Hz, 3 H, 6-CH_3), 1.23 (m, 2 H, 8-Et-CH_2), 1.59 (m, 3 H, 28-H), 1.61 (m, 1 H, 20-H), 1.68 (m, 1 H, 24-H), 1.75 (m, 1 H, 8-H), 1.89 (s, 3 H, 12-CH_3), 1.92 (s, 3 H, 4-CH_3), 2.02 (m, 1 H, 18-H), 2.05−2.14 (m, 2 H, 11-H_2), 2.05−2.14 (m, 1 H, 22-H_{ax}), 2.57 (ddd, J = 13.0, 4.5, 1.5 Hz, 1 H, 22-H_{eq}), 2.65 (m, 1 H, 6-H), 2.86 (m, 1 H, 10-H), 3.22 (s, 3 H, 16-OCH_3), 3.38 (m, 1 H, 21-H), 3.40 (m, 1 H, 25-H), 3.54 (m, 1 H, 19-H), 3.53 (s, 3 H, 2-OCH_3), 3.77 (br. d, J = 10.0 Hz, 1 H, 7-H), 3.81 (dd, J = 9.0, 9.0 Hz, 1 H, 16-H), 4.08 (ddd, J = 12.0, 12.0, 4.5 Hz, 1 H, 23-H), 5.05 (m, 1 H, 9-H), 5.07 (m, 1 H, 17-H), 5.21 (dd, J = 15.0, 9.0 Hz, 1 H, 15-H), 5.31 (m, 1 H, 26-H), 5.56 (dq, J = 15.0, 6.5 Hz, 1 H, 27-H), 5.62 (d, J = 10.0 Hz, 1 H, 5-H), 5.82 (br. d, J = 11.0 Hz, 1 H, 13-H), 6.39 (s, 1 H, 3-H), 6.59 (dd, J = 15.0, 11.0 Hz, 1 H, 14-H), 7.28 (d, J = 8.5 Hz, 2 H, 2 × 3′′H), 8.06 (d, J = 8.5 Hz, 2 H, 2 × 2′′H); ^{13}C NMR (125 MHz, CD_2Cl_2): δ = 8.2 (q, 20-CH_3), 9.6 (q, 18-CH_3), 12.1 (q, 8-Et-CH_3), 14.3 (q, 4-CH_3), 16.1 (q, 12-CH_3), 16.9 (q, 6-CH_3), 17.8 (q, C-28), 19.5 (q, 24-CH_3), 21.7 (q, 10-CH_3), 29.4 (t, 8-Et-CH_2), 34.1 (d, C-10), 35.3 (d, C-6), 37.3 (d, C-18), 38.7 (d, C-23), 39.5 (d, C-20), 45.4 (t, C-22), 46.3 (d, C-24), 44.5 (d, C-8), 45.9 (t, C-11), 55.9 (q, 16-OCH_3), 59.4 (q, 2-OCH_3), 69.6 (d, C-19), 75.1 (d, C-7), 76.1 (d, C-17), 80.5 (d, C-21), 81.4 (d, C-9), 82.4 (d, C-16), 84.2 (d, C-25), 121.4 (s, C-CF_3), 123.4 (s, CF_3), 124.2 (d, C-13), 126.9 (d, 2 × C-3′′),128.1 (d, C-15), 129.0 (d, C-27), 131.2 (d, C-26), 130.2 (d, 2 × C-2′′), 130.3 (d, C-3), 132.1 (s, C-4), 132.7 (s, C-1′′), 133.8 (d, C-14), 133.9 (s, C-4′′), 139.2 (d, C-5), 142.0 (s, C-2), 142.6 (s, C-12), 165.7 (s, COO) 166.2 (s, C-1); ESI MS: m/z = 1021 [M + Na] (pos.); $C_{48}H_{66}F_3IN_2O_9$ (998.98).

23-Iodo(^{125}I)-9-*O*-[*p*-(trifluoroethyldiazirinyl)benzoyl]-21,23-dideoxyconcanolide A (11b): Na^{125}I (10 mCi) was purchased as a solution in water. The water was removed in vacuo and **10** (13 mg, 0.012 mmol), NaI (4.5 mg, 0.030 mmol, 2.5 equiv.) and DMF (2 mL) were added to the residue. The resulting mixture was stirred for 48 h at 40 °C and the workup was carried out as described for **11a** to yield **11b** (5.9 mg, 50%) with a specific radioactivity of 438.7 mCi/mmol.

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