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Chemistry of Insect Antifeedants from *Azadirachta Indica* (Part 16):[1] Synthesis of Several Derivatives of Azadirachtin Containing Fluorescent or Immunogenic Reporter Groups.

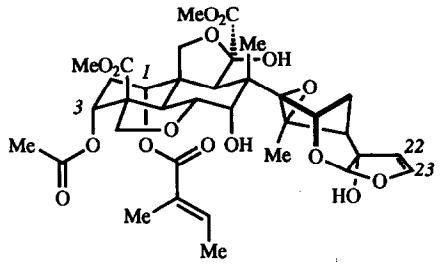
by Robert B. Grossman and Steven V. Ley*

Department of Chemistry, University Chemical Laboratories, Lensfield Road, Cambridge CB2 1EW, England

Abstract: Dinitrophenylamino, dansyl, and biotin groups have been covalently attached to several azadirachtin derivatives via a linker group to give fluorescent or immunogenic compounds that generally retain the biological properties of azadirachtin. These compounds are potential tools for the determination of the mechanisms of azadirachtin's action in living systems.

Azadirachtin (1) is a natural product whose remarkable insecticidal and other biological properties have received increasing attention in recent years.[2-4] Azadirachtin's structure features a

Azadirachtin (**1**)



densely packed array of oxygen functionalities of many different types, making its total synthesis a true challenge that has yet to be achieved.[2] The complex chemistry of azadirachtin has made it difficult to determine which portions of the molecule are responsible for its many biological effects,[3] including its recently discovered anti-malarial properties.[4] Furthermore, only the most cursory information about how azadirachtin exerts its effects in living systems is currently available.[3] If any further progress is to be made in understanding the biological mode of action of azadirachtin, the proteins or other cellular entities with which it interacts will have to be elucidated. In pursuit of this goal, we have undertaken the synthesis of a number of derivatives of azadirachtin that contain immunogenic or fluorescent reporter groups.[5] These derivatives generally retain the biological activity of azadirachtin, and the reporter groups which are attached to them provide a means of identifying azadirachtin-biological molecule

complexes *in vivo* or *in vitro*. The synthetic work described in this paper marks an important step towards unravelling the biochemical pathways that are responsible for the activity of this fascinating and important natural product.

We have focused our attention on attaching three particular reporter groups to azadirachtin (Figure 1). The dinitrophenylamino (DNP) group is highly absorbent of violet light, making a DNP-azadirachtin conjugate potentially useful for the development of a binding assay. Monoclonal and polyclonal antibodies to DNP are commercially available, allowing immunological methods for the *in vivo* determination of DNP-azadirachtin to be developed.[6] Commercially available immobilized monoclonal antibodies might also be used in combination with a DNP-azadirachtin conjugate to develop an affinity chromatography column. Another reporter group which we have investigated, the highly fluorescent dansyl group, has complementary properties to DNP. A dansyl-azadirachtin conjugate could conceivably be seen in a cell under a confocal microscope without the need for introducing any

Reporter groups used in this study

DNP	Dansyl	Biotin
highly colored immunogenic unnatural moderately polar	fluorescent unnatural moderately polar	ligand to avidin found in nature very polar

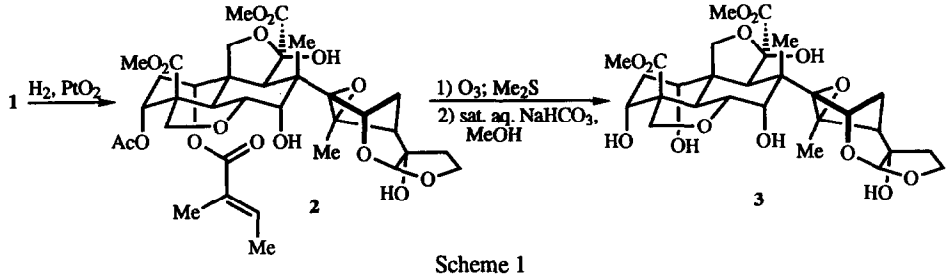
Figure 1

antibodies. It also might be used in a binding assay.[7] Immunohistological techniques involving the third reporter group, biotin, have been well explored, and a biotin-azadirachtin conjugate might also be used to develop an affinity chromatography column.[6,8] However, this group has the disadvantages of being naturally occurring and extremely polar, making it possible that a biotin-azadirachtin conjugate would behave more like biotin than like azadirachtin.

It remained to decide how to attach the reporter groups to azadirachtin. In the course of different studies on azadirachtin, we and others have had occasion to synthesize a number of derivatives of azadirachtin whose biological activities have shed light on the structure-activity relationships (SARs) in this compound.[2,9] The most consistent SAR that we have found is that there is not much change in the biological activity of azadirachtin when the acyl groups attached to the 1- and 3-hydroxyls are modified or removed. We have also found that the 3-hydroxyl of azadirachtin is readily saponified and re-esterified in good yield without concomitant rearrangement of the azadirachtin skeleton. For these reasons, we decided to attach the reporter groups to the 3-position in azadirachtin. For reasons of literature precedence[10] and commercial availability of the appropriate derivatives, we decided to use the 6-aminohexanoyl group as a linker to join the reporter group to the main body of the compound.

Results

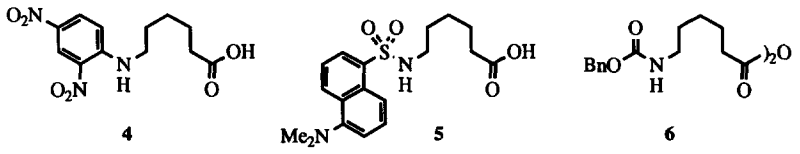
Using a slight modification of literature procedures, hydrogenation of **1** over PtO_2 under 1 atm H_2 gas gave 22,23-dihydroazadirachtin, **2** (Scheme 1).[9b,11] The ease of this reaction depended on the purity of **1**; this may account for an earlier observation that the reaction required several atmospheres of



Scheme 1

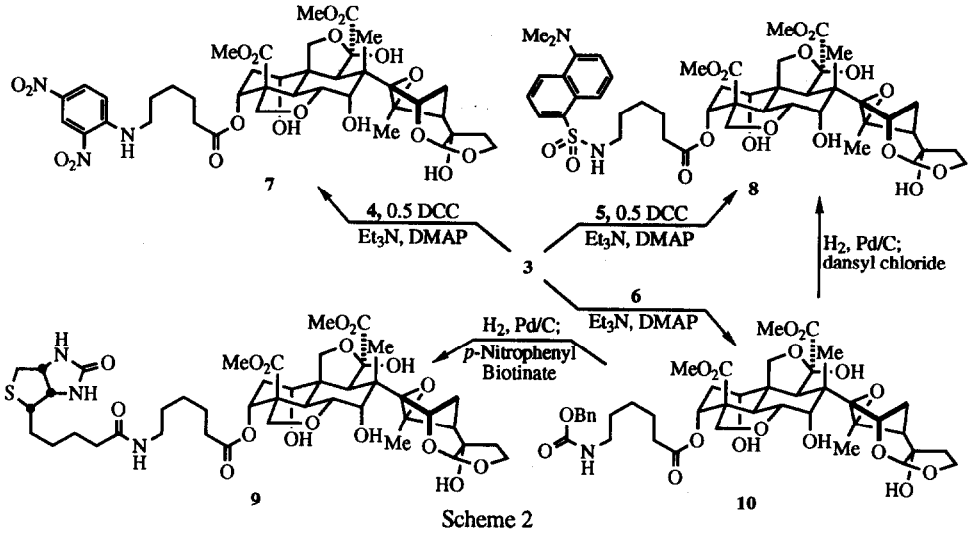
H_2 to proceed.[9b] Ozonolysis of the tiglate group followed by hydrolysis of the acetate and pyruvate esters gave the pentaol **3**, which showed good antifeedant activity against the African leafworm *S. littoralis*[12a] but, in contrast to the parent compound, failed to prevent the formation of motile male malaria gametes.[4,12b]

Acylation of **3** with the DNP-substituted acid[13a] **4** in the presence of dicyclohexylcarbodiimide (DCC) gave bright yellow **7** in 66% yield. This compound showed moderate antifeedant and growth regulatory activity against leafworms,[12a] very high activity against malaria gametes,[12b] and binding to locust testis[12c] that was comparable to **2**. Acylation of **3** with the dansyl-substituted acid **5**,[13a] under the same conditions that were used to prepare **7**, gave fluorescent compound **8** in a surprisingly low

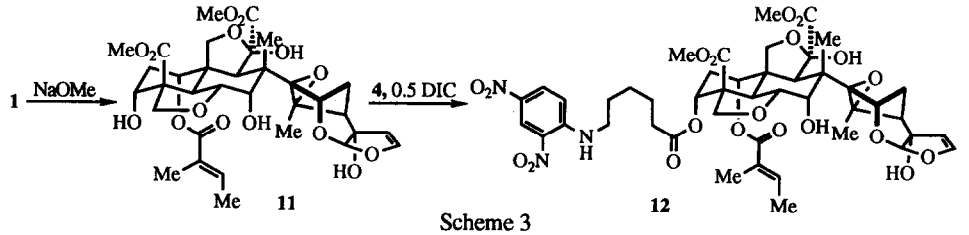


11% yield, with a 62% recovery of **3**. Alternatively, acylation of **3** with the Cbz-protected anhydride[10] **6** gave protected compound **10**, and hydrogenolysis of **10** followed by treatment with dansyl chloride gave **8** in 35% yield. On the other hand, hydrogenolysis of **10** followed by acylation with *p*-nitrophenyl biotinate[13b] gave **9** in 39% yield, 60% based on recovered starting material (Scheme 2). Compound **9**, unfortunately, showed no activity against either leafworms or malaria parasites.[12a,b]

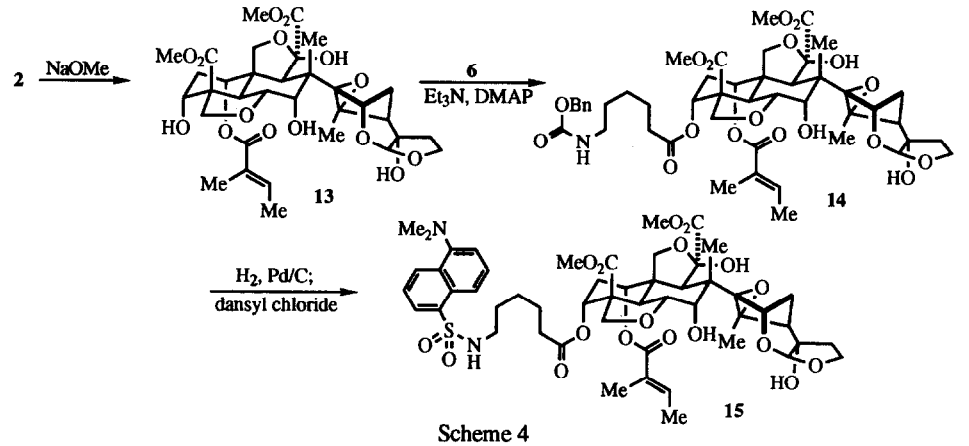
The antifeedant activities shown by compounds **7** and **9** were insufficiently high for the development of a binding assay.[12a] From our SAR data, we surmised that a compound that was less polar than the ones we had synthesized might show improved activity. We therefore decided to synthesize labelled compounds which retained, if possible, the enol ether double bond and the tigloyl group of **1**, although we were uncertain whether the presence of the large 1-tigloyl group would hinder acylation of the 3-hydroxyl. In order to synthesize a DNP-azadirachtin derivative, **1** was deacetylated



Scheme 2



Scheme 3



Scheme 4

under standard conditions to give **11** (Scheme 3).[9b,11,14] This was gratifyingly easily acylated with DNP acid **4** to give **12** in 58% yield. We found that **12** was easier to purify when 1,3-diisopropylcarbodiimide (DIC) was used instead of the more customary DCC. Compound **12** showed antifeedant activity and strength of binding to locust testis that was comparable to that of **1** itself.[12a,c]

Turning our attention to a less polar dansyl-azadirachtin derivative, we decided to forgo the direct acylation of **11** with **5**, since the acylation of **3** with **5** had proceeded poorly. Instead, **2** was deacetylated in 38% yield to give **13**, which was then re-acylated with **6** to give **14** in 79% yield (Scheme 4). Hydrogenolysis of **14** followed by treatment with dansyl chloride gave fluorescent compound **15** in 42% yield. Hydrogenation of the tiglate residue was not observed under these conditions.

We believe that compounds **7, 8, 9, 12,** and **15** will be used extensively in the determination of the mechanisms of action of azadirachtin. The results of these experiments[12] will be reported in due course.

Acknowledgments: We would like to thank Dr. M. S. J. Simmonds, Dr. J. Mordue, Prof. R. E. Sinden, Dr. R. H. C. Strang and their co-workers for their fruitful collaborative efforts and helpful suggestions. We would also like to thank Röhm and Haas and AgriDyne, Inc. for generous gifts of crude azadirachtin. We would like to thank the National Science Foundation for a NSF-NATO Post-Doctoral Fellowship and Ciba for further post-doctoral support (to RBG). We also acknowledge further financial support from Pfizer Central Research and the BP Research Endowment (to SVL).

Experimental Procedures

All solvents were distilled before use. Petroleum ether refers to the 40-60 °C fraction. Proton and carbon NMR spectra were obtained on a Bruker AM-400 spectrometer. HETCORR spectra were obtained on a Bruker DRX-500 spectrometer. The residual solvent peaks were used as internal reference. ($CDCl_3$: δ 7.25 [^{1}H] or 77.0 [^{13}C] ppm; CD_3OD: δ 3.30 [^{1}H] or 49.0 [^{13}C] ppm.) Infrared spectra were recorded on a Perkin-Elmer FTIR 1620 spectrometer. Mass spectra were recorded on a Kratos MS890MS spectrometer. Flash chromatography was conducted on Merck Kieselgel 60 silica (230-400 mesh). Crude (30%) azadirachtin was chromatographed once (70%, then 75% EtOAc/ petroleum ether) before use; crude (12%) azadirachtin required two chromatographic purifications (first time: 70%, then 75% EtOAc/ petroleum ether; second time: 3%, then 3.5% EtOH/ CH_2Cl_2). Dihydroazadirachtin was synthesized as reported previously,[9b,11] except that PtO_2 was used as catalyst under 1 atm H_2 gas.

Notes on the reporting of the ^{1}H and ^{13}C NMR spectra: Results from the ^{13}C APT spectra are recorded after each ^{13}C resonance in the parentheses as "e" (even number of H atoms attached to the carbon in question) or "o" (odd number of H atoms). The assignment of each ^{1}H or ^{13}C resonance is written after each resonance, after the semi-colon inside the parentheses. The standard numbering for azadirachtin is used.[2] In compounds where the tigloyl group is present, the carbon atoms of the 3-acyl group are numbered starting from 6'; in compounds where it is absent, they are numbered starting from

1'. In dansyl compounds, the naphthalene nucleus is numbered from 1" to 8". Assignments for compounds for which HETCORR spectra were not obtained were made by analogy to compounds **1, 2, 7,** and **9,** for which they were obtained.

***O*^{1}-Pyruvoyl-22,23-dihydroazadirachtin:**[11,15] The synthesis of this compound via ozonolysis was reported previously, but no experimental details were given.[15] A solution of **2** (2.75 g, 3.81 mmol) in methanol (ca. 200 mL) was cooled to -78 °C and subject to a stream of O_3 gas (180 V, 60 L/h). After 1.5 h, the ozonizer was shut off, and the solution was purged with O_2 gas until it was no longer blue. Then Me_2S (ca. 12 mL) was added, and the solution was allowed to warm to room temperature. The solvent was evaporated, and the residue was allowed to dry in vacuo overnight. This was subject to flash chromatography (eluant: 85% EtOAc/ petroleum ether). The white solid which was obtained was taken up in CH_2Cl_2, and the solvent was evaporated again to remove residual EtOAc. After drying in vacuo overnight, the title compound (2.02 g, 2.85 mmol, 75% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43 (t, 2.7 Hz, 1H; 3), 5.22 (s, 1H; 21), 4.95 (s, 1H; 11-OH), 4.70 (d, 2.6 Hz, 1H; 7), 4.64 (d, 3.3 Hz, 1H; 15), 4.57 (dd + m, $J_{d\ small}$= 2.7 Hz, 2H; 1, 6), 4.19 (d, 9.9 Hz, 1H; 19a), 4.06 (d, 9.0 Hz, 1H; 28a), 4.00 (m, 1H; 23a), 3.87 (m, 1H; 23b), 3.81 (d, 9.0 Hz, 1H; 28b), 3.78 (s, 3H; Me ester), 3.73 (s, 3H; Me ester), 3.58 (d, 9.9 Hz, 1H; 19b), 3.31 (s, 2H; 9, 20-OH), 3.23 (d, 12.5 Hz, 1H; 5), 2.74 (s, 1H; 7-OH), 2.46 (d, 5.1 Hz, 1H; 17), 2.45 (s, 3H; 5'), 2.37 (dt, J_d=16.9 Hz, J_t=2.5 Hz, 1H; 2a), 2.23 (dt, J_d=17.1 Hz, J_t=3.1 Hz, 1H; 2b), 2.12 (m, 2H; 22), 2.05 (s, 3H; 18), 2.02 (s, 3H; Ac), 1.73 (s, 3H; 30), 1.62 (m, 2H; 16). ^{13}C{^{1}H} NMR and APT (100 MHz, $CDCl_3$): δ 191.6 (e; 2'), 173.1 (e; 29), 171.7 (e; 12), 170.1 (e; Ac), 161.0 (e; 1'), 107.5 (o; 21), 104.6 (e; 11), 81.0 (e; 20), 76.5 (o; 15), 74.4 (o; 7), 73.7 (o; 6), 73.0 (e; 28), 72.5 (o; 1), 69.7 (e; 13 or 14), 69.2 (e; 19), 68.7 (e; 14 or 13), 66.7 (o; 3), 64.9 (e; 23), 53.3 (o; Me ester), 52.9 (o; Me ester), 52.5 (e; 4), 50.6 (o; 17), 50.0 (e; 10), 44.8 (e; 8), 44.4 (o; 9), 41.2 (e; 22), 36.7 (o; 5), 29.7 (e; 2), 26.4 (o; 5'), 24.3 (e; 16), 20.9 (o; 30), 20.9 (o; Ac), 18.1 (o; 18). HRMS (FAB): Calc. for [$C_{33}H_{42}O_{17}$ + Na]: 733.2319. Found: 733.2298.

***O*^{1}-Detigloyl-*O*^{3}-desacetyl-22,23-dihydroazadirachtin (3):** Compound **16** (2.38 g, ca. 85% pure, 2.85 mmol) was dissolved in methanol (40 mL), and sat. aq. $NaHCO_3$ (10 mL) was added. The following day, more sat. aq. $NaHCO_3$ (30 mL) was added. The following day, more sat. aq. $NaHCO_3$ (25 mL), methanol (40 mL), and water (10 mL) were added. After another 5 h, the mixture was diluted with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2 (3 × ca. 200 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to give a white solid. This was subject to flash chromatography (eluant: EtOAc). The white solid which was obtained was redissolved in CH_2Cl_2, and the solvent was evaporated again. After drying in vacuo overnight, **3** (585 mg [corrected for residual solvent], 0.98 mmol, 35% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.17 (s, 1H; 21), 4.62 (broad d, 2H; 7, 15), 4.55 (s, 1H; 11-OH), 4.51 (dd, 2.8 Hz, 12.5 Hz, 1H; 6), 4.33 (d, 9.9 Hz, 1H; 19a), 4.29 (t, 2.7 Hz, 1H; 3), 4.22 (d, 8.7 Hz, 1H; 28a), 4.04 (d, 8.6 Hz, 1H; 28b), 3.8-4.0 (m, 4H; 23, 1-OH, 3-OH), 3.80 (s, 3H; Me ester), 3.71 (s, 3H; Me ester), 3.57 (m, 1H; 1), 3.50 (v. broad, 1H; 20-OH), 3.41 (d, 9.8 Hz, 1H; 19b), 3.20 (s, 1H; 9), 3.04 (d, 12.5 Hz, 1H; 5), 2.86 (s, 1H; 7-OH), 2.43 (d,

5.0 Hz, 1H; 17), 2.21 (dt, J_d=15.7 Hz, J_t=3.0 Hz, 1H; 2a), 2.0-2.15 (m, 6H; 16a, 18 [δ 2.06], 22), 1.82 (d, partly obscured, ~16.6 Hz, 1H; 2b), 1.78 (s, 3H; 30), 1.64 (m, 1H; 16b). $^{13}C\{^1H\}$ NMR and APT (100 MHz, $CDCl_3$): δ 174.3 (e; 29), 173.4 (e; 12), 107.6 (o; 21), 104.3 (e; 11), 81.0 (e; 20), 76.5 (o; 15), 74.9 (o; 7), 73.9 (o; 6), 73.4 (e; 28), 71.0 (e; 19), 70.9 (o; 1), 69.6 (e; 13 or 14), 68.2 (e; 14 or 13), 67.2 (o; 3), 64.9 (e; 23), 54.9 (e; 4), 53.5 (o; Me ester), 52.5 (e; 10), 52.3 (o; Me ester), 50.4 (o; 17), 44.9 (e; 8), 44.4 (o; 9), 41.2 (e; 22), 32.9 (e; 2), 32.8 (o; 5), 24.3 (e; 16), 20.8 (o; 30), 18.7 (o; 18). HRMS (FAB): Calc. for [$C_{28}H_{38}O_{14}$ + Na]: 621.2159. Found: 621.2174.

***O*^{1}-Detigloyl-*O*^{3}-(6-[2,4-dinitrophenylamino]hexanoyl)-22,23-dihydroazadirachtin (7):** To a solution of **4** (256 mg, 861 μmol)[13a] in CH_2Cl_2 (ca. 30 mL) was added DCC (89 mg, 430 μmol). Some precipitate formed within 15 min. The reaction mixture was allowed to stir overnight. Then **3** (50 mg, 83 μmol), Et_3N (140 μL, 1.0 mmol), and a catalytic amount of DMAP were added. The suspension was allowed to stir for three days. It was filtered, then transferred to a separatory funnel and shaken with a mixture of 1 *N* HCl and brine. The aqueous layer was shaken further with two small portions of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated. The bright yellow residue was subject to flash chromatography (eluant: 90% EtOAc/ petroleum ether). The product was subject again to flash chromatography (eluant: 2.4%, then 3.2% EtOH/ CH_2Cl_2) to furnish **7** (46 mg, 52 μmol, 63% yield) as a bright yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ 9.12 (d, 2.5 Hz, 1H; 9'), 8.54 (broad, 1H; NH), 8.27 (dd, 2.4 Hz, 9.5 Hz, 1H; 11'), 6.91 (d, 9.5 Hz, 1H; 12'), 5.40 (s, 1H; 3), 5.18 (s, 1H; 21), 4.65 (broad, 2H; 7, 15), 4.60 (s, 1H; 11-OH), 4.56 (dd, 2.6 Hz, 12.6 Hz, 1H; 6), 4.16 (d, 9.5 Hz, 1H; 19a), 4.00 (d + m, J_d= 8.8 Hz, 2H; 23a, 28a), 3.87 (d + m, J_d= 8.8 Hz, 2H; 23b, 28b), 3.78 (s, 3H; Me ester), 3.75 (s, 3H; Me ester), 3.41 (m, 5H; 1, 1-OH or 20-OH, 19b, 6'), 3.29 (m, 1H; 9), 3.26 (s, 1H; 20-OH or 1-OH), 3.16 (d, 12.6 Hz, 1H; 5), 2.65 (s, 1H; 7-OH), 2.44 (d, 4.8 Hz, 1H; 17), 2.39 (q, 7.4 Hz, 2H; 2'), 2.30 (dm, J_d= 16.3 Hz, 1H; 2a), 2.0-2.2 (m, 6H; 2b, 18 [δ 2.06], 22), 1.5-1.9 (m, 11H; 16, 30 [δ 1.74], 3', 4', 5'). $^{13}C\{^1H\}$ NMR and APT (100 MHz, $CDCl_3$): δ 173.4 (e; 12), 173.2 (e; 29), 172.3 (e; 1'), 148.3 (e; 8', 10'), 136.1 (e; 7'), 130.4 (o; 11'), 124.4 (o; 9'), 113.9 (o; 12'), 107.6 (o; 21), 104.6 (e; 11), 81.0 (e; 20), 76.5 (o; 15), 74.8 (o; 7), 74.1 (o; 6), 72.9 (e; 28), 70.3 (e; 19), 69.6 (e; 13 or 14), 69.1 (o; 1), 68.1 (e; 14 or 13), 67.9 (o; 3), 64.8 (e; 23), 53.4 (o; Me ester), 52.9 (e; 4), 52.6 (o; Me ester), 52.0 (e; 10), 50.5 (o; 17), 44.8 (e; 8), 44.1 (o; 9), 43.4 (e; 6'), 41.3 (e; 22), 35.3 (o; 5), 34.3 (e; 2'), 31.5 (e; 2), 28.5 (e; 3' or 5'), 26.4 (e; 4'), 24.2 (e; 16, 5' or 3'), 21.0 (o; 30), 18.6 (o; 18). NMR assignments confirmed by HETCORR. IR (Nujol mull): 3444, 3366, 2922, 2854, 1731, 1621, 1462, 1336, 1040 cm^{-1}. HRMS (FAB): Calc. for [$C_{40}H_{51}N_3O_{19}$ + H]: 878.3194. Found: 878.3109.

***O*^{1}-Detigloyl-*O*^{3}-(6-[5-(dimethylamino)naphthalene-1-sulfonamido]hexanoyl)-22,23-dihydroazadirachtin (8):** The dicyclohexylammonium salt of **5** (365 mg, 669 μmol)[13a] was dissolved in dilute 1 *N* HCl. The solution was titrated with sat. aq. $NaHCO_3$ until it became very cloudy. The mixture was extracted with three portions of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated to give **5** as an oil. This was dissolved in CH_2Cl_2 and added to a

solution of DCC (69 mg, 334 μmol) in CH_2Cl_2 (ca. 40 mL). The reaction mixture was allowed to stir overnight in the dark. Then **3** (41 mg, 68 μmol), Et_3N (140 μL, 1.0 mmol), and a catalytic amount of DMAP were added. The suspension was allowed to stir for three days. A second portion of Et_3N (140 μL, 1.0 mmol) and DMAP were then added. The suspension was again allowed to stir for three days. The solvent was evaporated, and the residue was redissolved in a small volume of CH_2Cl_2, filtered, and evaporated again. The residue was subject to flash chromatography (eluant: EtOAc). Some **3** was recovered (ca. 28 mg, ca. 68% recovery), slightly contaminated with fluorescent material. On the other hand, the product which was obtained was subject again to flash chromatography (eluant: 3%, then 3.5% EtOH/ CH_2Cl_2). The product was subject a third time to flash chromatography (eluant: 90% EtOAc/ petroleum ether) to furnish **8** (7 mg, 7 μmol, 11% yield) as a fluorescent yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.53 (d, 8.5 Hz, 1H; 2"), 8.28 (d, 8.6 Hz, 1H; 4"), 8.23 (dd, 1.1 Hz, 7.3 Hz, 1H; 8"), 7.54 (m, 2H; 3", 7"), 7.18 (d, 7.4 Hz, 1H; 6"), 5.39 (~t, 1H; 3), 5.20 (t + s, 2H; 21, NH), 4.70 (~s, 1H; 7), 4.64 (s, 2H; 15, 11-OH), 4.58 (dd, 2.7 Hz, 12.6 Hz, 1H; 6), 4.17 (d, 9.4 Hz, 1H; 19a), 3.99 (d + m, $J_d \approx$ 8.9 Hz, 2H; 23a, 28a), 3.83 (d + ~q, $J_d \approx$ 9.0 Hz, 2H; 23b, 28b), 3.79 (s, 3H; Me ester), 3.75 (s, 3H; Me ester), 3.44 (d + m, $J_d \approx$ 9.6 Hz, 2H; 1, 19b), 3.28 (s, 1H; 1-OH or 20-OH), 3.22 (m, 2H; 5, 9), 2.88 (s, 8H; 6', Me_2N), 2.48 (d, 3.6 Hz, 1H; 17), 2.26 (m, 3H; 2a, 2'), 2.11 (m, 5H; 22, 18), 2.02 (m, 1H; 2b), 1.76 (s, 3H; 30), 1.55 (m, 2H; 16), 1.5-1.7 (m, 2H; 5' or 3'), 1.40 (quintet, 2H; 4'), 1.15-1.3 (m, 2H; 3' or 5'). Two OH resonances were not observed. $^{13}C\{^1H\}$ NMR and APT (100 MHz, $CDCl_3$): δ 173.4 (e; 12), 173.2 (e; 29), 172.1 (e; 1'), 152.0 (e; 5"), 134.8 (e; 1"), 130.4 (o), 130.0 (e; 4a" or 8a"), 129.7 (e; 8a" or 4a"), 129.5 (o), 128.3 (o), 123.2 (o), 118.8 (o), 115.2 (o), 107.5 (o; 21), 104.6 (e; 11), 81.0 (e; 20), 76.5 (o; 15), 74.9 (o; 7), 74.0 (o; 6), 72.9 (e; 28), 70.2 (e; 19), 69.7 (e; 13 or 14), 69.2 (o; 1), 68.2 (e; 14 or 13), 67.9 (o; 3), 64.5 (e; 23), 53.4 (o; Me ester), 53.0 (e; 4), 52.6 (o; Me ester), 52.0 (e; 10), 50.6 (o; 17), 45.4 (o; NMe_2), 44.9 (e; 8), 44.1 (o; 9), 43.0 (e, 6'), 41.3 (e; 22), 35.3 (o; 5), 34.5 (e; 2'), 31.6 (e; 2), 29.7 (e; 3' or 5'), 25.9 (e; 4'), 24.3 (e; 16), 24.0 (e; 5' or 3'), 21.1 (o; 30), 18.6 (o; 18). HRMS (FAB): Calc. for $[C_{46}H_{60}N_2O_{17}S + H]$: 945.3691. Found: 945.3658.

***O*^{1}-Detigloyl-*O*^{3}-(6-[benzyloxycarbonylamino]hexanoyl)-22,23-dihydroazadirachtin (10):** To a solution of **3** (110 mg, 184 μmol), Et_3N (220 μL, 1.6 mmol), and a catalytic amount of DMAP was added **6** (299 mg, 583 μmol).[10] The solution was allowed to stir for two days. It was diluted with EtOAc and shaken with sat. aq. $NaHCO_3$, then brine, and it was then dried over $MgSO_4$ and evaporated. After drying in vacuo overnight, a solid (289 mg) was obtained. This was subject to flash chromatography (eluant: EtOAc). The white solid that was obtained consisted mostly of **10,** but some **6** was still present. It was subject again to flash chromatography (eluant: 85% EtOAc/ petroleum ether). A white solid was obtained (109 mg, 129 μmol, 70% yield). ^1H NMR (400 MHz, $CDCl_3$): δ 7.30 (m, 5H; 10', 11', 12'), 5.40 (broad s, 1H; 3), 5.17 (s, 1H; 21), 5.05-5.20 (m, 3H; 8', NH), 4.71 (s, 1H; 11-OH), 4.66 (~d, 1H; 7), 4.63 (d, 2.6 Hz, 1H; 15), 4.56 (dd, 2.5 Hz, 12.6 Hz, 1H; 6), 4.16 (d, 9.4 Hz, 1H; 19a), 3.99 (d, 8.9 Hz, 1H; 28a), 3.92 (m, 1H; 23a), 3.84 (d, 8.9 Hz, 1H; 28b), 3.7-3.8 (m, 8H; 23b, Me ester [δ 3.77], Me ester [δ 3.74], 1-OH), 3.42 (d + s, 9.5 Hz, 2H; 19b, 1), 3.1-3.3 (m, 6H; 5, 9, 6', 7-OH, 20-OH), 2.41 (d, 4.4 Hz, 1H; 17), 2.34 (t, 7.5 Hz, 2H; 2'), 2.28 (d, 16.3 Hz, 1H; 2a), 1.9-2.1

(m, 6H;2b, 18 [δ 2.09], 22), 1.73 (s, 3H; 30), 1.64 (m, 3H; 16a, 3' or 5'), 1.58 (m, 1H; 16b), 1.50 (m, 2H; 4'), 1.35 (m, 2H; 5' or 3'). ^{1}H NMR (400 MHz, CD_3OD): δ 7.3-7.4 (m, 5H; 10', 11', 12'), 5.43 (s, 1H; 21), 5.37 (~t, 1H; 3), 5.05 (s, 2H; 8'), 4.75 (broad, 1H; 7), 4.51 (dd, 2.3 Hz, 12.5 Hz, 1H; 6), 4.33 (s, 1H; 15), 4.09 (d, 9.2 Hz, 1H; 19a), 3.97 (~q, 2H; 23), 3.90 (d, 8.6 Hz, 1H; 28a), 3.82 (d, 8.6 Hz, 1H; 28b), 3.75 (s, 3H; Me ester), 3.74 (s, 3H; Me ester), 3.53 (m, 2H; 1, 9), 3.44 (m, 2H; 5, 19b), 3.10 (t, 6.9 Hz, 2H; 6'), 2.40 (m, 3H; 17, 2'), 2.23 (m, 1H; 22a), 2.14 (s, 2H; 2), 2.00 (s, 3H; 18), 1.92 (m, 1H; 22b), 1.6-1.8 (m, 7H; 16, 30 [δ 1.63], 5'), 1.50 (m, 2H; 4'), 1.38 (m, 2H; 3'). $^{13}C\{^1H\}$ NMR and APT (100 MHz, $CDCl_3$): δ 173.4 (e; 12), 173.1 (e; 29), 172.2 (e; 1'), 156.6 (e; 7'), 136.5 (e; 9'), 128.5 (o; 10' or 11'), 128.1 (o; 11' or 10'), 107.4 (o; 21), 104.5 (e; 11), 80.8 (e; 20), 76.5 (o; 15), 74.8 (o; 7), 74.1 (o; 6), 72.9 (e; 28), 70.1 (e; 19), 69.7 (e; 13 or 14), 69.1 (o; 1), 68.3 (e; 14 or 13), 67.8 (o; 3), 66.7 (e; 8'), 64.5 (e; 23), 53.3 (o; Me ester), 52.9 (e; 4), 52.5 (o; Me ester), 51.9 (e; 10), 50.6 (o; 17), 45.0 (e; 8), 44.1 (o; 9), 41.2 (e; 22), 40.7 (e; 6'), 35.2 (o; 5), 34.7 (e; 2'), 31.5 (e; 2), 29.7 (e; 3' or 5'), 26.0 (e; 4'), 24.4 (e; 16), 24.1 (e; 5' or 3'), 21.1 (o; 30), 18.6 (o; 18). One aryl resonance (12') obscured. $^{13}C\{^1H\}$ NMR and APT (100 MHz, CD_3OD): δ 175.3 (e; 12 or 29 or 1'), 174.7 (e; 29 or 1' or 12), 174.6 (e; 1' or 12 or 29), 158.9 (e; 7'), 138.5 (e; 9'), 129.5 (o; 10' or 11'), 129.0 (o; 12'), 128.8 (o; 11' or 10'), 107.4 (o; 21), 106.0 (e; 11), 82.5 (e; 20), 79.1 (o; 7), 76.2 (o; 6), 75.9 (o; 15), 73.8 (e; 28), 71.4 (e; 13 or 14), 71.1 (e; 19), 70.3 (o; 1), 70.2 (e; 14 or 13), 69.1 (o; 3), 67.6 (e; 23), 67.4 (e; 8'), 59.5 (e; 4), 54.2 (e; 10), 53.3 (o; Me ester), 52.9 (o; Me ester), 51.1 (o; 17), 47.2 (e; 8), 46.1 (o; 9), 42.1 (e; 22), 41.6 (e; 6'), 36.6 (o; 5), 35.3 (e; 2'), 33.2 (e; 2), 30.6, 27.3, 26.7 (all e; 3', 4', 5'), 25.5 (e; 16), 22.1 (o; 30), 19.0 (o; 18). HRMS (FAB): Calc. for [$C_{42}H_{55}NO_{17}$ + H]: 846.3548. Found: 846.3510.

Alternative procedure for the synthesis of 8: To a solution of **10** (28 mg, 33 μmol) in EtOH (ca. 4 mL) under an atmosphere of argon was added acetic acid (3.0 μL, 170 μmol) and then 10% Pd/C (5.0 mg). A balloon containing hydrogen gas was connected, the argon atmosphere was purged with hydrogen, and the suspension was allowed to stir for 6 h. It was filtered through Celite, rinsing with EtOAc, and the solvent was evaporated. Some CH_2Cl_2 was added and evaporated, and finally the residue was dried in vacuo. An oil (34 mg) was obtained. This was redissolved in CH_2Cl_2 (ca. 6 mL). Dansyl chloride (18.2 mg, 67 μmol) and triethylamine (50 μL, 360 μmol) were added. The solution was allowed to stir overnight at room temperature. The solvent was evaporated, and the residue was subject to flash chromatography (eluant: 3.5%, then 4% EtOH/ CH_2Cl_2) to furnish **8** (11 mg, 12 μmol, 35% yield) as a fluorescent yellow solid.

***O*^{1}-Detigloyl-*O*^{3}-(6-[biotinamido]hexanoyl)-22,23-dihydroazadirachtin (9):** To a solution of **10** (51 mg, 60 μmol) in EtOH (8 mL) under Ar was added 10% Pd/C (3.1 mg) and AcOH (4.0 μL, 70 μmol). A balloon containing H_2 gas was attached, and the Ar atmosphere was purged with H_2. After 6 h, the reaction mixture was filtered through a plug of cotton, rinsing with EtOH. Then *p*-nitrophenyl biotinate[13b] (24 mg, 66 μmol) and Et_3N (30 μL, 220 μmol) were added. A yellow color developed soon. After 20 min, the solution was evaporated. The residue was dried in vacuo overnight and then subject to flash chromatography (eluant: EtOAc, followed by EtOH). Unreacted **10** (18 mg, 35%

recovery) was obtained from the EtOAc eluates. On the other hand, the EtOH eluates provided **9** (22 mg, 23 μmol, 39% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 5.46 (s, 1H; 21), 5.38 (s, 1H; 3), 4.75 (broad, 1H; 7), 4.50 (m, 2H; 6, 14'), 4.34 (s, 1H; 15), 4.30 (dd, 4.4 Hz, 7.8 Hz, 1H; 16'), 4.09 (d, 9.2 Hz, 1H; 19a), 3.99 (*AB*, 2H; 23), 3.91 (d, 8.6 Hz, 1H; 28a), 3.82 (d, 8.6 Hz, 1H; 28b), 3.76 (s, 3H; Me ester), 3.75 (s, 3H; Me ester), 3.55 (s, 1H; 1), 3.51 (s, 1H; 9), 3.45 (m, 2H; 5, 19b), 3.21 (m, 1H; 12'), 3.17 (t, 6.8 Hz, 2H; 6'), 2.93 (dd, 5.0 Hz, 12.7 Hz, 1H; 13'a), 2.70 (d, 12.7 Hz, 1H; 13'b), 2.43 (d, 5.4 Hz, 1H; 17), 2.39 (m, 2H; 2'), 2.26 (m, 1H; 22a), 2.19 (t, 7.3 Hz, 2H; 8'), 2.16 (s, 2H; 2), 1.99 (s, 3H; 18), 1.95 (m, 1H; 22b), 1.4-1.8 (m, 17H; 16, 30 [δ 1.63], 3', 4', 5', 9', 10', 11'). ^{13}C{^{1}H} NMR and APT (100 MHz, CD_3OD): δ 176.2, 175.5, 174.9, 174.7 (all e; 12, 29, 1', 15'), 166.2 (e; 7'), 107.5 (o; 21), 106.1 (e; 11), 82.6 (e; 20), 79.2 (o; 7), 76.3 (o; 6), 76.0 (o; 15), 74.0 (e; 28), 71.6 (e; 13 or 14), 71.3 (e; 19), 70.5 (o; 1), 70.5 (e; 14 or 13), 69.3 (o; 3), 67.8 (e; 23), 63.5 (o; 16'), 61.8 (o; 14'), 57.2 (o; 12'), 54.4 (e; 4), 53.5 (o; Me ester), 53.4 (e; 10), 53.1 (o; Me ester), 51.2 (o; 17), 47.4 (e; 8), 46.2 (o; 9), 42.3 (e; 22), 41.2 (e; 13'), 40.3 (e; 6'), 37.0 (e; 8'), 36.8 (o; 5), 35.4 (e; 2'), 33.4 (e; 2), 30.3, 30.0, 29.7, 27.7, 27.1, 26.9 (all e; 3', 4', 5', 9', 10', 11'), 25.6 (e; 16), 22.3 (o; 30), 19.2 (o; 18). NMR assignments confirmed by HETCORR. HRMS (FAB): Calc. for [$C_{44}H_{63}N_3O_{17}S$ + H]: 938.3956. Found: 938.3924.

***O*^{3}-Desacetylazadirachtin (11):**[9b,11,14] To a solution of **1** (202 mg, 280 μmol) in dry MeOH (20 mL) under Ar was added NaH (60% suspension in oil, 122 mg, 3.05 mmol). After 30 min, excess solid NH_4Cl was added. The solvent was evaporated, and the residue was resuspended in CH_2Cl_2, filtered, and evaporated again. The residue was subject to flash chromatography (eluant: 70% EtOAc/ petroleum ether). The slightly impure product was subject again to flash chromatography (eluant: 4% EtOH/ CH_2Cl_2) to furnish **11** (82 mg, 120 μmol, 43% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.84 (dq, J_d= 1.0 Hz, J_q=7.0 Hz, 1H; 3'), 6.44 (d, 2.9 Hz, 1H; 23), 5.65 (s, 1H; 21), 5.04 (d, 2.9 Hz, 1H; 22), 5.02 (s, 1H; 11-OH), 4.84 (~t, J< 1.0 Hz, 1H; 3), 4.74 (s, 1H; 7), 4.65 (d, 3.3 Hz, 1H; 15), 4.59 (dd, 2.6 Hz, 12.4 Hz, 1H; 6), 4.36 (m, 1H; 1), 4.25 (d, 8.7 Hz, 1H; 28a), 4.21 (d, 9.9 Hz, 1H; 19a), 4.06 (d, 8.7 Hz, 1H; 28b), 3.76 (s, 3H; Me ester), 3.68 (s, 3H; Me ester), 3.59 (d, 9.8 Hz, 1H; 19b), 3.29 (s, 1H; 9), 3.23 (d, 12.4 Hz, 1H; 5), 3.00 (broad, 1H; 7-OH), 2.80 (s, 1H; 20-OH), 2.37 (d, 5.3 Hz, 1H; 17), 2.1-2.3 (m, 3H; 2, 3-OH), 2.01 (s, 3H; 18), 1.84 (s, 3H; 5'), 1.78 (d, 7.0 Hz, 3H; 4'), 1.75 (s, 3H; 30), 1.69 (~dt, J_d≈ 12.9 Hz, 1H; 16a), 0.97 (d, 14.1 Hz, 1H; 16b). ^{13}C{^{1}H} NMR and APT (100 MHz, $CDCl_3$): δ 174.2 (e; 29), 171.8 (e; 12), 165.9 (e; 1'), 147.0 (23), 138.2 (o; 3'), 128.5 (e; 2'), 108.8 (o; 22), 107.5 (o; 21), 104.3 (e; 11), 83.5 (e; 20), 76.4 (o; 15), 74.5 (o; 7), 73.8 (o; 6), 73.0 (e; 28), 71.9 (o; 1), 69.9 (e; 13 or 14), 69.5 (e; 19), 68.8 (e; 14 or 13), 66.1 (o; 3), 54.0 (e; 4), 53.3 (o; Me ester), 52.5 (o; Me ester), 50.4 (e; 10); 48.8 (o; 17), 45.3 (e; 8), 44.8 (o; 9), 35.4 (o; 5), 32.1 (e; 2), 25.2 (e; 16), 21.2 (o; 30), 18.4 (o; 18), 14.5 (o; 4'), 12.1 (o; 5'). HRMS (FAB): Calc. for [$C_{33}H_{42}O_{15}$ + H]: 679.2601. Found: 679.2571. Calc. for [$C_{33}H_{42}O_{15}$ + Na]: 701.2421. Found: 701.2432.

***O*^{3}-(6-[2,4-dinitrophenylamino]hexanoyl)azadirachtin (12):** To a solution of **4** (178 mg, 600 μmol)[13a] in dry CH_2Cl_2 (ca. 40 mL) under Ar was added 1,3-diisopropylcarbodiimide (47 μL, 300

μmol). The reaction mixture was allowed to stir overnight. Then **11** (32 mg, 47 μmol), Et_3N (140 μL, 1.0 mmol), and a catalytic amount of DMAP were added. The suspension was allowed to stir for three days, and it was then evaporated. The bright yellow residue was subject to flash chromatography (eluant: 75% EtOAc/ petroleum ether). The desired product was contaminated with diisopropylurea, so it was dissolved in a small amount of CH_2Cl_2 and filtered, rinsing the solid with ether until it was no longer yellow. The filtrate was evaporated and subject again to flash chromatography (eluant: 2.8% EtOH/ CH_2Cl_2). The product was twice taken up in CH_2Cl_2 and evaporated to remove residual EtOH. After drying in vacuo overnight, **12** (26 mg, 27 μmol, 58% yield) was obtained as a bright yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ 9.13 (d, 2.6 Hz, 1H; 14'), 8.52 (broad, 1H; NH), 8.27 (dd, 2.6 Hz, 9.4 Hz, 1H; 16'), 6.92 (dq + d', J_d= 9.4 Hz, J_q= 6.4 Hz, 2H; 3', 17'), 6.45 (d, 2.9 Hz, 1H; 23), 5.62 (s, 1H; 21), 5.52 (~t, J<1.0 Hz, 1H; 3), 5.04 (d, 2.9 Hz, 1H; 22), 5.03 (s, 1H; 11-OH), 4.75 (s, 1H; 7), 4.73 (s, 1H; 1), 4.66 (d, 3.2 Hz, 1H; 15), 4.59 (dd, 2.6 Hz, 12.5 Hz, 1H; 6), 4.13 (d, 9.7 Hz, 1H; 19a), 4.06 (d, 8.9 Hz, 1H; 28a), 3.79 (s, 3H; Me ester), 3.71 (d, 8.9 Hz, 1H; 28b), 3.66 (s, 3H; Me ester), 3.62 (d, 9.7 Hz, 1H; 19b), 3.41 (t, 6.9 Hz, 1H; 11'a), 3.39 (t, 6.9 Hz, 1H; 11'b), 3.30 (d + s, J_d= 12 Hz, 2H; 5, 9), 2.85 (broad, 2H; 7-OH, 20-OH), 2.37 (d, 5.2 Hz, 1H; 17), 2.1-2.3 (m, 4H; 2, 7'), 1.99 (s, 3H; 18), 1.83 (s, 3H; 5'), 1.6-1.8 (m, 11H; 16a, 30 [δ 1.73], 4' [δ 1.77, d, 7.0 Hz], 8', 10'), 1.41 (~quintet, ~7 Hz, 2H; 9'), 1.30 (d, 13.1 Hz, 1H; 16b). $^{13}C\{^1H\}$ NMR and APT (100 MHz, $CDCl_3$): δ 173.3 (e; 29), 172.0 (e; 6'), 171.8 (e; 12), 166.2 (e; 1'), 148.2 (e; 15'), 147.2 (e + o; 23, 13'), 137.5 (o; 3'), 136.1 (e; 12'), 130.4 (o; 14'), 128.7 (e; 2'), 124.3 (o; 16'), 113.8 (o; 17'), 108.8 (o; 22), 107.4 (o; 21), 104.1 (e; 11), 83.6 (e; 20), 76.5 (o; 15), 74.3 (o; 7), 73.8 (o; 6), 72.9 (e; 28), 70.6 (o; 1), 70.0 (e; 13 or 14), 69.0 (e; 19), 68.5 (e; 14 or 13), 67.0 (o; 3), 53.3 (o; Me ester), 52.8 (o; Me ester), 52.5 (e; 4), 50.2 (e; 10); 48.6 (o; 17), 45.5 (e; 8), 44.7 (o; 9), 43.2 (e; 11'), 37.1 (o; 5), 33.8 (e; 7'), 29.9 (e; 2), 28.4 (e; 8' or 10'), 26.4 (e; 9'), 25.0 (e; 16), 24.2 (e; 10' or 8'), 21.3 (o; 30), 18.4 (o; 18), 14.3 (o; 4'), 12.0 (o; 5'). HRMS (FAB): Calc. for [$C_{45}H_{55}N_3O_{20}$ + H]: 958.3457. Found: 958.3478. Calc. for [$C_{45}H_{55}N_3O_{20}$ + H - H_2O]: 940.3351. Found: 940.3436.

***O*^{3}-Desacetyl-22,23-dihydroazadirachtin (13):** A solution of **2** (200 mg, 277 μmol) in dry methanol (ca. 20 mL) under Ar was treated with NaH (60% suspension in mineral oil, 122 mg, 3.05 mmol). The mixture was allowed to stir for 30 min, when it was treated with an excess of solid NH_4Cl. The solvent was evaporated; the residue was taken up in CH_2Cl_2, filtered, and evaporated again. The residue was subject to flash chromatography (eluant: 4.5% EtOH/ CH_2Cl_2). The product which was obtained was subject twice more to flash chromatography (eluant: EtOAc). The product was taken up in CH_2Cl_2, and the solvent was evaporated to remove residual EtOAc. After drying in vacuo overnight, **13** (72 mg, 106 μmol, 38% yield) was obtained as a white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 6.81 (dq, J_d= 1.4 Hz, J_q= 7.0 Hz, 1H; 3'), 5.25 (s, 1H; 21), 5.02 (s, 1H; 11-OH), 4.82 (t, 2.7 Hz, 1H; 3), 4.71 (d, 2.6 Hz, 1H; 7), 4.65 (d, 3.3 Hz, 1H; 15), 4.58 (dd, 2.7 Hz, 12.4 Hz, 1H; 6), 4.36 (m, 1H; 1), 4.22 (m, 2H; 19a, 28a), 4.05 (d, 8.7 Hz, 1H; 28b), 4.00 (m, 1H; 23a), 3.90 (m, 1H; 23b), 3.75 (s, 3H; Me ester), 3.68 (s, 3H; Me ester), 3.59 (d, 8.5 Hz, 1H; 19b), 3.26 (s, 1H; 9), 3.18 (m, 2H; 5, 20-OH), 2.79 (s, 1H; 7-OH), 2.45 (d, 5.2 Hz, 1H; 17), 2.1-2.25 (m, 5H; 2, 22, 3-OH), 2.01 (s, 3H; 18), 1.84 (s, 3H; 5'), 1.78

(dd, 1.0 Hz, 7.0 Hz, 3H; 4'), 1.75 (s, 3H; 30), 1.68 (m, 1H; 16a), 1.51 (d, 12.8 Hz, 1H; 16b). $^{13}C\{^1H\}$ NMR and APT (100 MHz, $CDCl_3$): δ 174.2 (e; 29), 171.7 (e; 12), 165.9 (e; 1'), 138.1 (o; 3'), 128.5 (e; 2'), 107.3 (o; 21), 104.3 (e; 11), 81.2 (e; 20), 76.7 (o; 15), 74.5 (o; 7), 73.8 (o; 6), 73.0 (e; 28), 71.8 (o; 1), 69.6 (e; 13 or 144), 69.5 (e; 19), 68.6 (e; 14 or 13), 66.0 (o; 3), 65.1 (e; 23), 54.0 (e; 4), 53.3 (o; Me ester), 52.5 (o; Me ester), 50.4 (o; 17), 50.2 (e; 10), 45.1 (e; 8), 44.8 (o; 9), 41.3 (e; 22), 35.5 (o; 5), 32.1 (e; 2), 24.5 (e; 16), 21.1 (o; 30), 18.4 (o; 18), 14.5 (o; 4'), 12.1 (o; 5'). HRMS (FAB): Calc. for [$C_{33}H_{44}O_{15}$ + H]: 681.2758. Found: 681.2758.

***O*³-(6-[benzyloxycarbonylamino]hexanoyl)-22,23-dihydroazadirachtin (14):** To a solution of **13** (62 mg, 91 μmol) in CH_2Cl_2 (ca. 8 mL) was added **6** (184 mg, 359 μmol),[10] Et_3N (80 μL, 570 μmol), and a few crystals of DMAP. The reaction mixture was allowed to stir overnight. The solvent was evaporated, and the residue was subjected to flash chromatography (eluant: 3%, then 3.2% EtOH/ CH_2Cl_2). The white foam which was obtained was subject again to flash chromatography (eluant: 3%, then 3.2% EtOH/ CH_2Cl_2). The product was dried in vacuo overnight to give **14** (67 mg, 72 μmol, 79% yield) as a white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.31 (m, 5H; 15', 16', 17'), 6.89 (~q, ~6.8 Hz, 1H; 3'), 5.49 (s, 1H; 3), 5.29 (s, 1H; 21), 5.19 (m, 3H; 13', 11-OH), 4.84 (m, 1H; NH), 4.72 (s, 2H; 1, 7), 4.66 (d, 3.2 Hz, 1H; 15), 4.57 (dd, 12.5 Hz, 2.5 Hz, 1H; 6), 4.14 (d, 9.7 Hz, 1H; 19a), 4.04 (d, 8.9 Hz, 1H; 28a), 3.98 (m, 1H; 23a), 3.86 (m, 1H; 23b), 3.77 (s, 3H; Me ester), 3.66 (s + d, 4H; 28b, Me ester), 3.60 (d, 9.7 Hz, 1H; 19b), 3.38 (s, 1H; 20-OH), 3.35 (m, 2H; 5, 9), 3.17 (m, 2H; 11'), 3.07 (s, 1H; 7-OH), 2.45 (d, 4.9 Hz, 1H; 17), 2.1-2.3 (m, 5H; 2, 22a, 7'), 2.02 (s + m, 4H; 18, 22b), 1.83 (s, 3H; 5'), 1.76 (d, 6.9 Hz, 3H; 4'), 1.74 (s, 3H; 30), 1.45-1.7 (m, 6H; 16, 8', 10'), 1.30 (m, 2H; 9'). $^{13}C\{^1H\}$ NMR and APT (100 MHz, $CDCl_3$): δ 173.4 (e; 29), 172.2 (e; 6'), 171.8 (e; 12), 166.3 (e; 1'), 156.7 (12'), 137.4 (e; 14'), 136.4 (o; 3'), 128.8 (e; 2'), 128.5 (o; 17', 15' or 16'), 128.1 (o; 16' or 15'), 107.2 (o; 21), 104.3 (e; 11), 81.2 (e; 20), 76.7 (o; 15), 74.3 (o; 7), 73.9 (o; 6), 73.0 (e; 28), 70.6 (o; 1), 69.7 (e; 13 or 14), 69.2 (e; 19), 68.6 (e; 14 or 13), 66.9 (o + e; 3, 13'), 65.2 (e; 23), 53.2 (o; Me ester), 52.7 (o; Me ester), 52.6 (e; 4), 5.4 (o; 17), 50.1 (e; 10), 45.3 (e; 8), 44.7 (o; 9), 41.4 (e; 22), 40.7 (e; 11'), 37.2 (o; 5), 34.3 (e; 7'), 30.0 (e; 2), 29.6 (e; 8' or 10'), 26.2 (e; 9'), 24.6 (e; 16, 10' or 8'), 21.4 (o; 30), 18.6 (o; 18), 14.3 (o; 4'), 12.0 (o; 3'). HRMS (FAB): Calc. for [$C_{47}H_{61}NO_{18}$ + H]: 928.3967. Found: 928.4022.

***O*³-(6-[5-(dimethylamino)naphthalene-1-sulfonamido]hexanoyl)-22,23-dihydroazadirachtin (15):** To a solution of **14** (67 mg, 72 μmol) in ethanol (ca. 8 mL) under an atmosphere of argon was added 10% Pd/C (11 mg, 10 μmol) and acetic acid (10 μL, 570 μmol). A balloon of hydrogen gas was attached, and the argon atmosphere was purged with hydrogen. The suspension was allowed to stir vigorously at room temperature. After 3.5 h, TLC showed that no **14** remained. The suspension was filtered through Celite, rinsing with ethanol, and the filtrate was evaporated. Ethyl acetate was added; a white precipitate formed. The solvent was evaporated. Then CH_2Cl_2 was added and evaporated. The white solid that was obtained was dissolved in CH_2Cl_2 (ca. 15 mL). Dansyl chloride (39 mg, 145 μmol) and triethylamine (1.05 mL, 0.75 mmol) were added. The solution was allowed to stir for 4.5 h, whereupon it was evaporated. After drying in vacuo for 2 h, the material was subjected to flash chroma-

tography (eluant: 3%, then 3.2% EtOH/ CH_2Cl_2). Impure fractions were resubmitted to flash chromatography in the same solvent. The combined product was taken up in CH_2Cl_2, and the solvent was evaporated to remove residual EtOH. The residue was dried in vacuo overnight to give **15** (31 mg, 30 μmol, 42% yield) as a fluorescent yellow solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.53 (d, 8.5 Hz, 1H; 2"), 8.24 (d, 8.6 Hz, 1H; 4"), 8.21 (d, 7.3 Hz, 1H; 8"), 7.52 (m, 2H; 3", 7"), 7.17 (d, 8.6 Hz, 1H; 6"), 6.93 (q, 7.0 Hz, 1H; 3'), 5.48 (s, 1H; 3), 5.13 (s + broad, 2H; 21, NH), 5.09 (s, 1H; 11-OH), 4.72 (s, 2H; 1,7), 4.63 (d, 3,2 Hz, 1H; 15), 4.58 (dd, 12.5 Hz, small *J*, 1H; 6), 4.12 (d, 9.7 Hz, 1H; 19a), 4.04 (d, 8.8 Hz, 1H; 28a), 3.91 (dt, J_d= 4.5 Hz, J_t= 8.5 Hz, 1H; 23a), 3.77 (s, 3H; Me ester), 3.66 (m, 5H; 23b, 28b, Me ester [δ 3.66]), 3.61 (d, 9.6 Hz, 1H; 19b), 3.34 (s, 1H; 20-OH), 3.33 (d, 12.5 Hz, 1H; 5), 3.29 (m, 2H; 9, 7-OH), 2.87 (s + m, 8H; 11', NMe_2), 2.43 (d, 5.1 Hz, 1H; 17), 2.25 (m, 2H; 2), 2.0-2.2 (m, 3H; 7', 22a), 2.00 (s, 3H; 18), 1.98 (m, 1H; 22b), 1.82 (s, 3H; 5'), 1.77 (d, 6.9 Hz, 3H; 4'), 1.72 (s, 3H; 30), 1.3-1.7 (m, 6H; 16 [δ 1.64 (ddd), 1.51 (d, 13.0 Hz)], 8', 10'), 1.21 (m, 2H; 9'). $^{13}C\{^1H\}$ NMR and APT (100 MHz, $CDCl_3$): δ 173.4 (e; 29), 172.3 (e; 6'), 171.8 (e; 12), 166.3 (e; 1'), 152.1 (e; 5"), 137.8 (o; 3'), 134.7 (e; 1"), 130.5 (o), 130.0 (e), 129.6 (e), 129.4 (o), 128.6 (e; 2'), 128.4 (o), 123.2 (o), 118.7 (o), 115.2 (o), 107.0 (o; 21), 104.5 (e; 11), 81.1 (e; 20), 76.7 (o; 15), 74.3 (o; 7), 73.9 (o; 6), 73.0 (e; 28), 70.6 (o; 1), 69.7 (e; 13 or 14), 69.1 (e; 19), 68.6 (e; 14 or 13), 66.9 (o; 3), 64.8 (e; 23), 53.2 (o; Me ester), 52.8 (o; Me ester), 52.5 (e; 4), 50.3 (o; 17), 50.2 (e; 10), 45.4 (o; 8, NMe_2), 44.7 (o; 9), 42.9 (e; 11'), 41.3 (e; 22), 37.2 (o; 5), 33.9 (e; 7'), 30.0 (e; 2), 29.3 (e; 8' or 10'), 25.7 (e; 9'), 24.2 (e; 16), 24.2 (e; 10' or 8'), 21.4 (o; 30), 18.6 (o; 18), 14.4 (o; 4'), 12.0 (o; 5'). HRMS (FAB): Calc. for [$C_{51}H_{66}N_2O_{18}S$ + H]: 1027.4109. Found: 1027.4069.

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