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Inhibitory Effects on Mitochondrial Complex I of Semisynthetic Mono-Tetrahydrofuran Acetogenin Derivatives

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Abstract—Modifications in the terminal α,β-unsaturated γ-methyl-γ-lactone moiety or in the alkyl chain that links this terminal γ-lactone with the α,α′-dihydroxylated THF system of the natural mono-tetrahydrofuranic acetogenins, annonacin and annonacinone, led to the preparation of eight semisynthetic derivatives. Their inhibitory effects on mitochondrial complex I is discussed and compared with that of the classical complex I inhibitor, rotenone.

Over 400 acetogenins from Annonaceae have been isolated and characterized so far. This class of natural products shows remarkable biological properties, for example, as antitumor, antiparasitic, inmunosuppressant or pesticidal agents.[1] Recently, it has been described that several acetogenins inhibit proliferation of human tumor cell lines by inducing apoptosis.[2] All these cytotoxic effects have been related with a specific inhibition on the enzyme NADH/ubiquinone oxidoreductase, or complex I, of the respiratory chain,[3–8] mainly acting at the level of the PSST subunit of the complex I, where the energy coupling redox reaction occur. Therefore, some acetogenins were found to be more potent inhibitors of this enzyme than the classic complex I inhibitors, piericidin A and rotenone.

Most of the annonaceous acetogenins are characterized by two functional units: an hydroxylated mono-THF or bis-THF ring system in the core of a long hydrocarbon chain and an α,β-unsaturated γ-lactone ring at one end of the alkyl chain.[1] Although several structure–activity relationship studies of acetogenins for complex I inhibition have been carried out,[4,9] the important structural factors have not been fully defined yet. Nevertheless, both units and the alkyl spacer between them could play significant roles in binding interactions with the enzyme, although it is possible to substitute the terminal γ-lactone with a quinone group or by a carboxylic acid without loss of activity.[9i]

Recently, we studied the structure–activity relationship of adjacent and non-adjacent bis-THF acetogenins through a series of semisynthetic derivatives with different functional groups at the α,α′-dihydroxylated THF system, the spacer alkyl chain that links the γ-lactone moiety with the THF system,[9g] or at the terminal γ-lactone.[9h] Some of these derivatives were found to be more potent complex I inhibitors than the parent natural acetogenins. In the present study, such modifications were carried out on mono-THF acetogenins in an attempt to examine if they follow the same or rather different trends than those observed for the bis-THF acetogenins.

Chemistry

Eight semisynthetic derivatives were prepared from two natural α,α′-dihydroxylated mono-THF acetogenins: annonacin (**1**) and annonacinone (**2**). They have identical *threo*/*trans*/*threo* relative configuration at the THF system and only differ by the functional group at the

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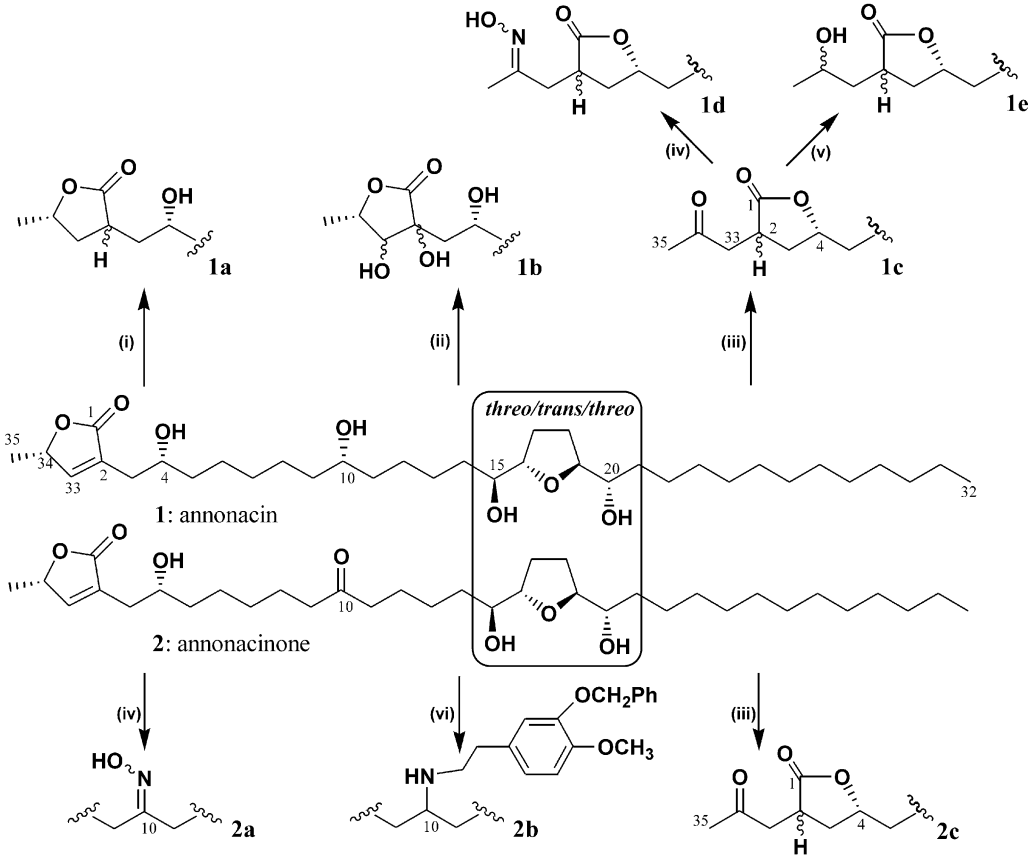
C-10 position, a hydroxyl group for annonacin (**1**) and a ketonic group for annonacinone (**2**). Annonacin (**1**) was isolated in a large amount from the seeds of *Annona muricata*[1] and *Annona glabra*[10] and it was used as starting material to prepare compounds **1a**–**1e** (Scheme 1). In all these compounds modifications have been carried out at the α,β-unsaturated γ-lactone moiety.

As previously described for other acetogenins,[9h,11,12] 2,33-dihydroannonacin (**1a**)[13] was obtained as a 1:1 mixture of two diastereoisomers (2,34-*cis* and 2,34-*trans*) by catalytic hydrogenation of **1** over 10% Pd/C. 2,33-Dihydro-2,33-dihydroxy annonacin (**1b**) was prepared in good yield by oxidation of **1** with osmium tetroxide and *N*-methylmorpholine *N*-oxide, a reagent described as producing a *cis*-hydroxylation.[14] The ^{1}H and ^{13}C NMR spectra of **1b** revealed double signal resonances for all the positions affecting the γ-lactone moiety.[15] Therefore **1b** must be considered as a mixture of the corresponding *cis*-dihydroxylated derivatives. The translactonized 2-acetonyl saturated γ-lactone derivatives (**1c** and **2c**)[16] were obtained as a 2,4-*cis* and 2,4-*trans* mixture under standard alkaline (diethylamine) treatment of a methanol solution of compounds,[17] and further purification by preparative HPLC at the specific wavelength of 263 nm. The carbonyl of the acetonyl group of **1c** is suitable for being transformed into the hydroxylimine derivative (**1d**)[18] by treatment with $H_2NOH.HCl$ in pyridine or to be reduced in the presence of $NaBH_4$ to obtain the desired 34-dihydrogenated derivative (**1e**).[19]

In an attempt to produce modifications at the alkyl chain that links the tetrahydrofuranic system of the molecule with the terminal α,β-unsaturated lactone, annonacinone (**2**), isolated from the seeds of *Annona glabra*,[10] was used as starting material to prepare compounds **2a**–**2b**. In these compounds modifications have been directed to introduce different nitrogenated substituents at the C-10 carbonyl group of annonacinone: a oxyme group (**2a**)[20] or the bulky nitrogenated group, such as in **2b**[21] (Scheme 1) through chemical transformations that do not affect the very sensitive α,β-unsaturated γ-methyl-γ-lactone located at the terminus. The interest in these compounds is based on previous results obtained for several nitrogenated derivatives (oxyme, triazido, amino or *N*-formyl amino) prepared from bis-THF acetogenins (guanacone and squamocin) that proved how the presence of oxyme groups enhanced the potency of such compounds as complex I inhibitors, whereas the other derivatives only retain some activity.[9g]

Biological Evaluation

The natural acetogenins (**1**,**2**), the compounds **1a**–**1e**, and **2a**–**2c** were tested as inhibitors of beef heart mitochondrial



Scheme 1. Reagents and conditions: (i) H_2, 10% Pd/C, 1 atm, rt, 2 h; (ii) NMO/OsO_4, rt, 48 h, followed by saturated $NaHSO_3$, rt, 1 h; (iii) DEA, reflux, 24 h; (iv) $H_2NOH{\cdot}HCl/Pyr$, reflux, 3 h; (v) $NaBH_4/MeOH$, rt, 6 h; (vi) β-(3-(benzyloxy)-4 methoxy-phenyl) ethylamine/titanium (IV) isopropoxide, rt, 48 h, followed by sodium cyanoborohydride, rt, 20 h.

Table 1. Inhibitory potency of acetogenins and derivatives[a]

Compd	IC_{50} (nM)		Relative potency[b]	Relative potency[c]
	NADH oxidase	NADH/DB oxidoreductase		
1	2.3±0.3	26.1±3.2	3.2	0.62
1a	3.6±0.4	19.9±0.2	5.1	0.70
1b	21.8±2.5	55.2±13.1	31.1	4.27
1c	3.3±0.6	14.6±1.0	4.7	0.64
1d	1.9±0.1	8.7±2.27	2.7	0.37
1e	5.8±1.4	51.0±1.4	8.2	1.10
2	0.8±0.1	3.7±0.3	1.1	0.15
2a	2.7±0.5	10.3±1.2	3.8	0.52
2b	>150	>2000	N.D.	N.D.
2c	0.7±0.1	1.1±0.3	1.0	0.13
Rotenone	5.1±0.9	28.8±1.5	7.2	1.00

N.D., not determined.
[a]All assays were carried out at a final concentration of beef-heart submitochondrial particles of 6 μg/mL. Data are means±SD from four determinations.
[b]Relative potency compared with the NADH oxidase IC_{50} value of the most potent compound (**2c**).
[c]Relative potency compared with the NADH oxidase IC_{50} value of the compound of reference, rotenone.

complex I. Beef-heart submitochondrial particles were prepared as described previously.[22,23] The inhibitory potency was evaluated against both the integrated NADH oxidase activity and the specific complex I NADH/ubiquinone oxidoreductase activity using decylubiquinone (DB) as artificial ubiquinone-like substrate (NADH/DB oxidoreductase activity) at a constant protein concentration of submitochondrial particles in the assay.[9h,22] In Table 1, are summarized the inhibitory potencies of the tested compounds in terms of IC_{50} value, which is the molar concentration required to reduce the control enzymatic activities by 50%.

Although it has been reported that mono-THF acetogenins are less potent compounds than the adjacent and the non adjacent bis-THF acetogenins, our results have shown that all the tested compounds, with the exception of **1b** and **2b**, can block the electron transfer when complex I operates in physiological conditions (NADH oxidase activity) because they exhibited activities in the nanomolar range similar to the known strong inhibitor rotenone and other acetogenins. In addition, some of these compounds (**1**, **1a**, **1c**, **1d**, **2**, **2a**, and **2c**) were most effective to inhibit the NADH/DB oxidoreductase than the compound of reference (rotenone).

Nevertheless, the structure–activity relationship found for these compounds differs to that described for bis-THF acetogenins. In fact, only the oxyme derivative **1d**, as occurred for the same class of derivatives from rolliniastatin-1 and cherimolin-1,[9h] was found to be more potent than the parent acetogenin (**1**). Variations in the inhibitory potency of compounds **1a**, **1b**, **1c**, and **1e** were not comparable to those observed for similar γ-lactone derivatives prepared from rolliniastatin-1 and cherimolin-1,[9h] showing a decrease in inhibitory potency. From results obtained for **1b** (IC_{50} 21.8 and 55.2 nM against NADH oxidase and NADH/DB oxidoreductase, respectively) it is not possible to rule out the influence of its higher polarity. It has been suggested that approximately three hydroxyl groups seem be optimal for an appropriate diffusion of acetogenins across the biological membrane and reaching the transmembrane part of the enzyme.[5,24] Thus, in compound **1b**, due to the presence of five hydroxyl groups the access of the compound to the enzyme binding site could be limited.

As far as annonacinone derivatives are concerned, the translactonization gives a compound (isoannonacinone, **2c**) with a similar potency to that of annonacinone (**2**) against the NADH oxidase activity, but it is also the most potent compound found in this study as inhibitor of the specific NADH/DB oxidoreductase activity. It is interesting to note the large decrease of potency for compound **2a** (contrary to that found for 10-oxyme guanacone),[9g] and the lost of activity when a bulky and rigid nitrogenated group is placed at the C-10 position (compound **2b**) of the spacer moiety.

In conclusion, we have semisynthesized mono-THF acetogenin derivatives from annonacin and annonacinone and evaluated their ability to inhibit the mitochondrial complex I. The compounds **1d** and **2c** were more potent inhibitors than the parent compounds. Considering the data and previous studies, it is very likely that both the THF system of the core of the molecule and the terminal lactone be, simultaneously, implicated in binding the active site of the enzyme, with the relevant contribution of the spacer chain between them to appropriately orient both parts.

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13. Semisynthesis of 2,33-dihydroannonacin (**1a**). Annonacin (**1**, 23 mg, 0.038 mmol) in EtOH (30 mL) was treated with 10% Pd/C and H_2. The reaction was run at room temperature and under atmospheric pressure for 2 h. The obtained solution was filtered through a bed of Celite and concentrated in vacuum to afford **1a** in quantitative yield. Relevant values: $C_{35}H_{66}O_7$; $[\alpha]_D$ +3.3° (*c* 0.3, EtOH); IR ν_{max} 1746 cm^{-1}; LSIMS *m/z*: 621 $[M+Na]^+$, 599 $[MH]^+$; EIMS *m/z* (%): 449 (5), 381 (11), 363 (13), 345 (9), 337 (14), 319 (9), 311 (100), 293 (27), 281 (27), 275 (15), 269 (39), 263 (15), 251 (15), 243 (25), 225 (40), 207 (16), 199 (21), 197 (45), 169 (29), 143 (37), 125 (54), 113 (40); ^1H NMR* ($CDCl_3$, 400 MHz) δ 4.53 and 4.40 (2m, H-34 *cis* and *trans*), 3.87 and 3.64 (2m, H-4 *cis* and *trans*), 2.88 and 2.55 (2m, H-2 *cis* and *trans*), 2.55, 1.98, 1.55 (3m, H-3 and H-33), 1.42 and 1.30 (2d, $J=6.0$ Hz and $J=6.4$ Hz, H-35 *cis* and *trans*); ^{13}C NMR* ($CDCl_3$, 100 MHz) δ 180.1 (C-1), 79.6 and 75.8 (C-34 *cis* and *trans*), 70.2 and 66.5 (C-4 *cis* and *trans*), 41.5 and 39.2 (C-2 *cis* and *trans*), 39.6 and 35.3 (C-3 *cis* and *trans*), 36.0 (C-33), 24.1 and 20.7 (C-35 *cis* and *trans*). *The assignments were made by COSY, DEPT, HMQC, and HMBC.

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15. Semisynthesis of 2,33-dihydro-2,33-dihydroxyannonacin (**1b**). To a solution of annonacin (**1**, 100 mg, 0.169 mmol) and *N*-methyl morpholine *N*-oxide (NMO) (100 mg, 0.83 mmol) in EtOAc (4 mL) and water (2 mL) was added a 4% aqueous solution of osmium tetroxide (1000 μL, 0.190 mmol) dropwise. The mixture was stirred at room temperature for 48 h and then a saturated $NaHSO_3$ solution (6 mL) was added. After being stirred for 1 h at room temperature, the reaction mixture was partitioned between EtOAc/H_2O. The organic solutions were washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH 95:5) affording **1b** (73 mg, 67%). Relevant values: $[\alpha]_D$ +25.4° (*c* 0.3, EtOH); IR ν_{max} 1763 cm^{-1}; HRLSIMS *m/z*: 631.477832 $[MH]^+$ (calcd 631.478509 for $C_{35}H_{67}O_9$); EIMS *m/z* (%): 612 (3), 594 (2), 576 (3), 558 (2), 449 (6), 413 (8), 395 (16), 385 (9), 377 (11), 367 (22), 359 (6), 349 (8), 343 (95), 325 (38), 307 (21), 281 (11), 269 (100), 263 (8), 257 (9), 251 (21), 239 (13), 175 (11), 157 (6), 109 (35); ^1H NMR* ($CDCl_3$, 300 MHz) δ 4.68 and 4.08 (2m, H-4), 4.36 and 3.92 (2m, H-34), 3.79 (m, H-33), 2.26, 2.19, 2.09 and 1.75 (4dd, $J=6.1$ Hz and $J=14.6$ Hz, H-3), 1.46 and 1.30 (2d, $J=6.3$ Hz and 6.0 Hz, H-35); ^{13}C NMR* ($CDCl_3$, 75 MHz) δ 177.2 and 175.2 (C-1), 80.2 and 75.0 (C-2), 79.6 and 69.5 (C-34), 79.2 and 68.6 (C-4), 78.0 and 73.2 (C-33), 40.5 and 36.3 (C-3), 20.2 and 18.0 (C-35). * The assignments were made by COSY, DEPT, TOCSY, HMQC, and HMBC.

16. Semisynthesis of 2,4-*cis* and 2,4-*trans*-isoacetogenins (**1c** and **2c**). Annonacin (**1**, 200 mg, 0.335 mmol) was dissolved in methanol (10 mL) containing 64 μL of diethylamine and refluxed for 24 h. Water was added and the reaction mixture was extracted with CH_2Cl_2. The organic solutions were washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by semipreparative HPLC with acetonitrile/water 75:25 (flow rate 3 mL/min, $\lambda=263$ nm), to afford **1c** as a 3:1 mixture of 2,4 *cis* and 2,4 *trans* isomers (168 mg, 80%). Relevant values: $C_{35}H_{64}O_7$; $[\alpha]_D$ +26° (*c* 0.29, EtOH); IR ν_{max} 1765, 1715 cm^{-1}; LSIMS *m/z*: 619 $[M+Na]^+$, 597 $[MH]^+$; EIMS *m/z* (%): 397 (3), 379 (12), 361 (33), 309 (100), 291 (45), 281 (14), 269 (16), 263 (6), 241 (22), 141 (7); ^{13}C NMR* ($CDCl_3$, 100 MHz) δ 205.8 (C-34 *trans*), 205.6 (C-34 *cis*), 178.8 (C-1 *trans*), 178.2 (C-1 *cis*), 79.3 (C-4 *cis*), 78.8 (C-4 *trans*), 44.1 (C-33 *trans*), 43.7 (C-33 *cis*), 36.6 (C-2 *cis*), 35.4 (C-3 *cis*), 34.4 (C-2 *trans*), 33.3 (C-3 *trans*), 29.9 (C-35 *trans*), 29.8 (C-35 *cis*). For ^1H NMR, see ref 1a. *The assignments were made by COSY, DEPT, HMQC, and HMBC. **Compound 2c**: This compound was prepared in 81% yield from **2** (100 mg, 0.168 mmol) by the procedure described above for **1c**. Purification by column chromatography (CH_2Cl_2/MeOH 98:2) gave 80.2 mg of **2c**. Relevant values: $C_{35}H_{62}O_7$; $[\alpha]_D$ +20° (*c* 0.6, EtOH); IR ν_{max} 1766, 1715 cm^{-1}; LSIMS *m/z*: 617 $[M+Na]^+$, 595 $[MH]^+$; ^{13}C NMR* ($CDCl_3$, 75 MHz) δ 210.7 (C-10), 205.6 (C-34), 178.6 (C-1 *trans*), 178.2 (C-1 *cis*), 79.1 (C-4 *cis*), 78.7 (C-4 *trans*), 44.1 (C-33 *trans*), 43.6 (C-33 *cis*), 42.6 and 42.5 (C-9, 11), 36.6 (C-2 *cis*), 35.4 (C-3 *cis*), 34.4 (C-2 *trans*), 33.2 (C-3 *trans*), 29.9 (C-35 *trans*), 29.8 (C-35 *cis*). For ^1H NMR, see ref 1a. *The assignments were made by COSY, DEPT, HMQC, and HMBC.

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18. Semisynthesis of 34-deoxo-34-oxymeisoannonacin (**1d**).

Pyridine (130 μL) and hydroxylamine hydrochloride (130 mg, 1.87 mmol) were added to a EtOH solution (10 mL) of isoanonacin (**1c**, 33 mg, 0.055 mmol). After the mixture was stirred and refluxed for 3 h, water was added and the reaction mixture was extracted with CH_2Cl_2. The organic solutions were washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by flash column chromatography (CH_2Cl_2/EtOAc/MeOH 50:50:7.5) yielding **1d** as a mixture of *cis* and *trans* stereoisomers (25 mg, 75%). Relevant values: $[\alpha]_D + 23°$ (*c* 0.3, EtOH); HRLSIMS *m/z*: 612.483364 $[MH]^+$ (calcd 612.483929 for $C_{35}H_{66}O_7N$); ^1H NMR* ($CDCl_3$, 400 MHz) δ 4.50 (m, H-4 *trans*), 4.35 (m, H-4 *cis*), 3.02–2.98 (m, H-2 *trans*), 2.94-2.87 (m, H-2 *cis*), 2.81 (dd, *J* = 3.8 Hz and 15.9 Hz, H-33b *cis*), 2.69 (m, H-33b *trans*), 2.52–2.46 (m, H-3b *cis*), 2.33 (dd, *J* = 10.0 Hz and *J* = 15.6 Hz, H-33a *trans*), 2.27 (dd, *J* = 10.0 Hz and *J* = 15.9 Hz, H-33a *cis*), 2.09–2.02 (m, H-3b *trans*), 1.90 (s, H-35 *trans*), 1.87 (s, H-35 *cis*), 1.59–1.35 (m, H-3a); ^{13}C NMR* ($CDCl_3$, 100 MHz) δ: 178.7 (C-1 *trans*), 178.3 (C-1 *cis*), 155.4 (C-34 *cis*), 155.2 (C-34 *trans*), 79.1 (C-4 *cis*), 78.9 (C-4 *trans*), 38.2 (C-2 *cis*), 36.6 (C-2 *trans*), 36.5, 35.3 and 32.9 (C-3, C-5 and C-33 *trans*), 36.4, 35.2 and 35.1 (C-3, C-5 and C-33 *cis*), 13.9 (C-35 *cis*), 13.8 (C-35 *trans*). *The assignments were made by COSY, DEPT, HMQC, and HMBC.

19. Semisynthesis of 34-dihydroisoannonacin (**1e**). $NaBH_4$ (42 mg, 1.10 mmol) was added to a dry methanol solution (6 mL) of isoannonacin (**1c**, 40.7 mg, 0.068 mmol). After the mixture was stirred at room temperature for 6 h, a 5% methanol HCl solution was added to eliminate the excess of $NaBH_4$ and then extracted with H_2O/CH_2Cl_2. The organic solutions were washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was purified by semipreparative HPLC with acetonitrile/water 70:30 (flow rate 1.5 mL/min,λ = 235 nm), to afford **1e** as a mixture of two stereoisomers (34 mg, 84%). Relevant values: $[\alpha]_D + 11.4°$ (*c* 1.5, EtOH); IR (dry film) ν_{max} 1756 cm^{-1}; HRLSIMS *m/z*: 599.487655 $[MH]^+$ (calcd 599.488680 for $C_{35}H_{67}O_7$); EIMS *m/z* (%): 381 (9), 363 (14), 353 (22), 345 (16), 327 (14), 311 (100), 299 (17), 293 (36), 283 (95), 281 (12), 275 (17), 269 (21), 265 (35), 251 (12), 247 (17), 243 (34), 225 (31), 143 (20), 125 (39); ^1H NMR* ($CDCl_3$, 400 MHz) δ 4.52 and 4.38 (2m, H-4 *cis* and *trans*), 4.06 and 3.87 (2m, H-34 *cis* and *trans*), 2.95–2.79 (m, H-2 *cis* and *trans*), 2.53 and 1.55 (2m, H-3), 1.92 and 1.60 (2m, H-33), 1.72 and 1.50 (2m, H-5), 1.41 and 1.37 (2d, *J* = 6.8 Hz and *J* = 6.4 Hz, H-35 *cis* and *trans*); ^{13}C NMR* ($CDCl_3$, 100 MHz) δ 180.5 and 180.1 (C-1 *cis* and *trans*), 79.5 and 79.3 (C-4 *cis* and *trans*), 66.3 and 66.1 (C-34 *cis* and *trans*), 40.0 (C-33), 39.1 and 37.6 (C-2 *cis* and *trans*), 36.0 and 35.9 (C-3 *cis* and *trans*), 35.3 (C-5), 21.0 and 20.8 (C-35 *cis* and *trans*). *The assignments were made by COSY, DEPT, TOCSY, HMQC, and HMBC.

20. Semisynthesis of 10-deoxo-10-oxymeannonacinone (**2a**). This compound was prepared in 77% yield from **2** (47 mg, 0.074 mmol) by the procedure described above for **1d**. Purification by flash column chromatography (CH_2Cl_2/EtOAc/MeOH 50:40:4) yielded **2a** (*cis* and *trans* mixture, 38.7 mg). $[\alpha]_D + 22.3°$ (*c* 1.3, EtOH). HREIMS *m/z* (%): 609.460972 $[M]^+$ (calcd 609.460454 for $C_{35}H_{63}O_7N$), 591.4472 (calcd 591.4498 for $C_{35}H_{61}O_6N$, 4), 575.4541 (calcd 575.4549 for $C_{35}H_{61}O_5N$, 37), 573.4377 (calcd 573.4393 for $C_{35}H_{59}O_5N$, 17), 557.4352 (calcd 557.4444 for $C_{35}H_{59}O_4N$, 9), 410.2548 (calcd 410.2542 for $C_{22}H_{36}O_6N$, 25), 394.2607 (calcd 394.2593 for $C_{22}H_{36}O_5N$, 100), 392.2491 (calcd 392.2436 for $C_{22}H_{34}O_5N$,12), 376.2480 (calcd 376.2487 for $C_{22}H_{34}O_4N$, 19), 340.2121 (calcd 340.2123 for $C_{18}H_{30}O_5N$, 51), 324.2176 (calcd 324.2174 for $C_{18}H_{30}O_4N$, 91), 322.2042 (calcd 322.2018 for $C_{18}H_{28}O_4N$, 10), 306.2066 (calcd 306.2069 for $C_{18}H_{28}O_3N$, 22), 269.2486 (calcd 269.2480 for $C_{17}H_{33}O_2$, 30), 238.1458 (calcd 238.1443 for $C_{13}H_{20}O_3N$, 6), 211.1333 (calcd 211.1334 for C_{12} $H_{19}O_3$, 4), 141.0573 (calcd 141.0551 for $C_7H_9O_3$, 7); ^1H NMR* ($CDCl_3$, 400 MHz) δ 7.19 (d, *J* = 1.2 Hz, H-33), 5.04 (dq, *J* = 6.8 Hz and *J* = 1.2 Hz, H-34), 3.81 (m, H-4), 3.78 (m, H-16, 19), 3.40 (m, H-15, 20), 2.48 (dd, *J* = 15.2 Hz and *J* = 4.0 Hz, H-3a), 2.38 (dd, *J* = 15.2 Hz and *J* = 8.0 Hz, H-3b), 2.31 and 2.16 (2brt, *J* = 6.6 Hz and *J* = 6.8 Hz, H-9, 11 *cis* and *trans*), 1.98, 1.63 (2m, H-17, 18), 1.50 (m, H-8, 12), 1.46–1.24 (3m, H-5-7, H-13,14 and H-21-31), 1.41 and 1.25 (2d, *J* = 6.8 Hz and *J* = 6.4 Hz, H-35 *cis* and *trans*), 0.87 (t, *J* = 6.4 Hz, H-32). ^{13}C NMR* ($CDCl_3$, 100 MHz) δ 174.6 (C-1), 161.7 (C-10), 151.8 (C-33), 131.0 (C-2), 82.8 (C-19), 82.6 and 82.5 (C-16 *cis* and *trans*), 77.9 (C-34), 74.0 (C-20), 73.7 and 73.6 (C-15 *cis* and *trans*), 69.7 and 69.5 (C-4 *cis* and *trans*), 37.1 (C-5), 33.7, 33.5, 33.3, 33.2 and 33.0 (C-3,9,11,14,21), 31.8 (C-30), 29.7-29.3 (C-7 and 23-29), 28.7 (C-17,18), 25.9-25.1 (C-6, 8, 12, 13, 22), 22.6 (C-31), 19.0 (C-35), 14.1 (C-32). *The assignments were made by COSY, DEPT, HMBC, and HMBC.

21. Semisynthesis of 10-deoxo-10β-(3-(benzyloxy)-4-methoxyphenyl) ethylaminoannonacinone (**2b**). A mixture of annonacinone (**2**, 41.7 mg, 0.070 mmol), β-(3-(benzyloxy)-4-methoxyphenyl) ethylamine (0.081 mmol), and titanium (IV) isopropoxide (0.101 mmol) was stirred at room temperature. After 48 h, when the IR spectrum of the mixture showed no ketone band, the solution was diluted with ethanol. Sodium cyanoborohydride (0.054 mmol) was added, and the solution stirred for 20 h. Water was added with stirring, and the resulting inorganic precipitate was filtered and washed with ethanol. The filtrate was extracted with ethyl acetate, and the organic solutions were filtered and concentrated in vacuum. After purification by flash column chromatography (CH_2Cl_2/EtOAc/MeOH/NH_4OH 30:50:10:0.1) compound **2b** (18 mg, 30%) was obtained as a mixture of two diastereoisomers. $C_{51}H_{81}O_8N$; $[\alpha]_D$ −11.6° (*c* 0.6, EtOH). LSIMS *m/z*: 858 $[M+Na]^+$, 836 $[MH]^+$, 818 $[MH-H_2O]^+$, 745 $[MH-CH_2Ph]^+$; ^1H NMR* ($CDCl_3$, 400 MHz) δ 7.28–7.45 and 6.8 (8ArH), 7.20 (d, *J* = 1.2 Hz, H-33), 5.09 (s, OCH_2), 5.04 (dq, *J* = 6.8 Hz and *J* = 1.2 Hz, H-34), 3.83 (s, OCH_3), 3.81 (m, H-4), 3.78 (m, H-16, 19), 3.38 (m, H-15, 20), 3.04 (m, H-α), 3.00 (m, H-10), 2.98 (m, H-β), 2.47 (dd, *J* = 16 Hz and *J* = 1.6 Hz, H-3b), 2.37 (dd, *J* = 16 Hz and *J* = 8 Hz, H-3a), 1.97 and 1.63 (2m, H-17, 18), 1.52-1.31 (3m, H-5, 9, 11, 14, 21), 1.40 (d, *J* = 6.8 Hz, H-35), 1.24 (m, H-6-8, 12, 13, 22-31), 0.87 (t, *J* = 6.8 Hz, H-32); ^{13}C NMR* ($CDCl_3$, 100 MHz) δ 174.6 (C-1), 151.9 (C-33), 148.8 and 148.4 (C-3′ and 4′), 136.9 (C-1″), 131.0 (C-2), 129.7 (C-1′), 128.4 (C-3″, 5″), 127.8 (C-4″), 127.5 (C-2″, 6″), 121.5 (C-6′), 114.2 (C-2′), 112.8 (C-5′), 82.8 and 82.5 (C-16, 19), 78.0 (C-34), 74.1 (C-20), 73.7 and 73.6 (C-15), 71.0 (OCH_2), 69.7 and 69.5 (C-4), 58.2 and 57.9 (C-10), 56.1 (OCH_3), 36.9 and 36.4 (C-9, 11), 33.4 and 33.2 (C-3, 5), 32.8 and 32.7 (C-14, 21), 31.9 (C-30), 29.7-29.3 (C-7, 23-29), 28.7 (C-17, 18), 25.6-25.0 (C-6, 8, 12, 13, 22), 22.6 (C-31), 19.0 (C-35), 14.1 (C-32).* The assignments were made by COSY, DEPT, HMBC, and HMBC.

22. For biological methods, see ref 9g and references cited herein.

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