SYNTHESES OF DEXAMETHASONE CONJUGATES OF THE PHYTOHORMONES GIBBERELLIN A_3 AND 24-EPICASTASTERONE

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The syntheses of *N*-[10-(9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamido)decyl]gibberellamide (**7**) and 6-[({*N*-[10-(9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamido)decyl]carbamoyl}methoxy)imino]-24-epicastasterone (**10**) are described. [(Benzotriazol-1-yl)oxy]bis(pyrrolidin-1-yl)methylium hexafluorophosphate (HBPyU) was used as the coupling agent for the reaction of gibberellic acid as well as of 24-epicastasterone-*O*-(carboxymethyl)oxime with *N*-(10-aminodecyl)-9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamide (**4**). The gibberellic acid conjugate **7** was also synthesised by the coupling of succinimidyl gibberellate **6** with amine **4**.

Keywords: Phytohormones; Gibberellins; Steroids; Brassinosteroids; Conjugation; Dexamethasone; Gibberellin A_3; 24-Epicastasterone.

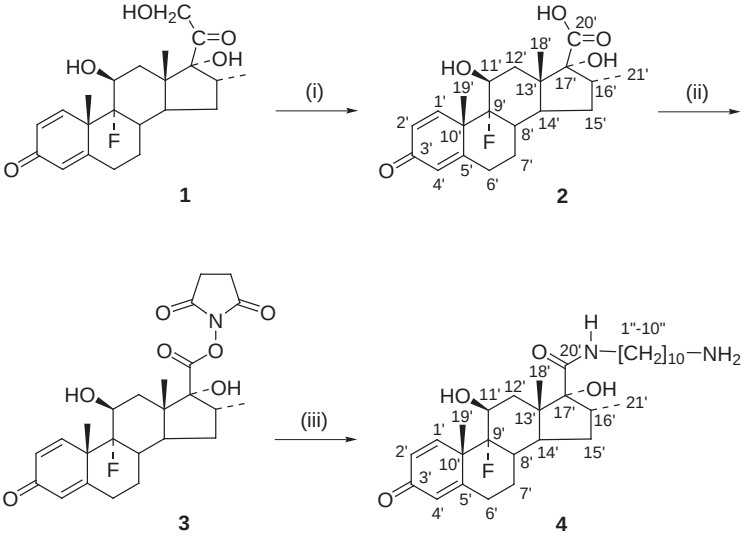
Heterodimer hybrid molecules derived from several natural products are useful tools in molecular biology to study the regulation of protein–protein interactions, to trigger signal transduction pathways and for the detection of ligand–protein receptor interactions[1–3]. As first models in such direction in the field of phytohormones, we describe herein the syntheses of heterodimers of gibberellin A_3 (ref.[4]) and the brassinosteroid 24-epicastasterone[5] having dexamethasone as the conjugated moiety.

RESULTS AND DISCUSSION

For the syntheses of phytohormone conjugates coupled with dexamethasone (9α-fluoro-16α-methylprednisolone) *via* the decane-1,10-diamine

spacer, dexamethasone (**1**) was oxidised in the first step with periodic acid. The obtained 9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid[6,7] (**2**) was converted into the succinimidyl ester **3** by the reaction with *N*-hydroxysuccinimide using dicyclohexylcarbodiimide as the coupling agent. The active ester **3** was treated with decane-1,10-diamine to afford *N*-(10-aminodecyl)-9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamide[2] (**4**) (Scheme 1), a suitable component for the coupling with the phytohormone gibberellin A_3 (**5**) as well as 24-epicastasterone (**8**). Compounds **2**, **3**, and **4** have been described[2,6,7], but without detailed spectroscopic data.

Starting from GA_3 (**5**), the corresponding succinimidyl ester was prepared by the reaction with *N*-hydroxysuccinimide using dicyclohexylcarbodiimide as the coupling agent. The obtained active ester **6** was treated with amine **4** to give, after repeated column chromatography and in a poor yield (16%), *N*-[10-(9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamido)decyl]gibberellamide (**7**). In a more efficient procedure, compound **7** was obtained in 57% yield by the coupling of **5**



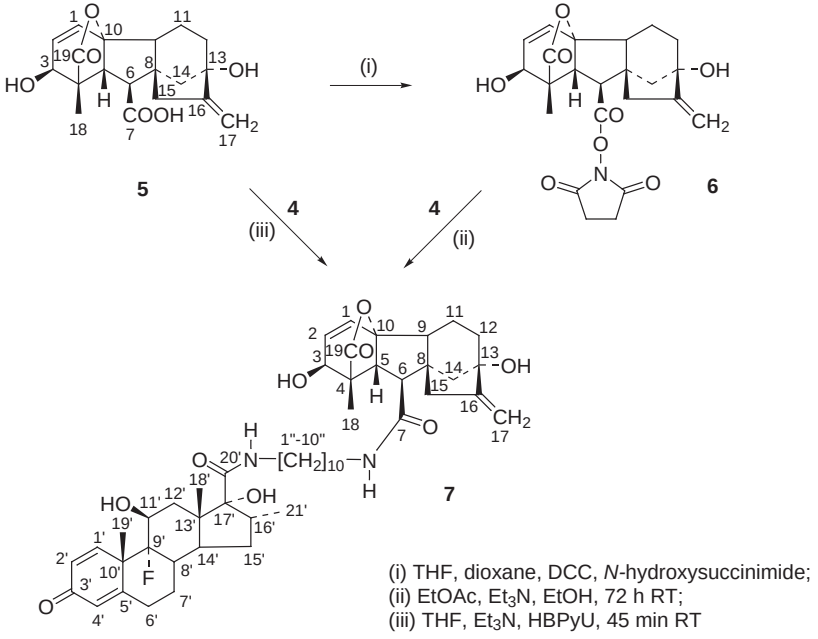
(i) EtOH, H_5IO_6,H_2SO_4; (ii) THF, dioxane, DCC, *N*-hydroxysuccinimide;
(iii) CH_2Cl_2, decane-1,10-diamine

SCHEME 1
Synthesis of *N*-(10-aminodecyl)-9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamide (**4**)

with **4** using the excellent peptide coupling agent [(benzotriazol-1-yl)-oxy]bis(pyrrolidin-1-yl)methylium hexafluorophosphate[8,9] (HBPyU) (Scheme 2).

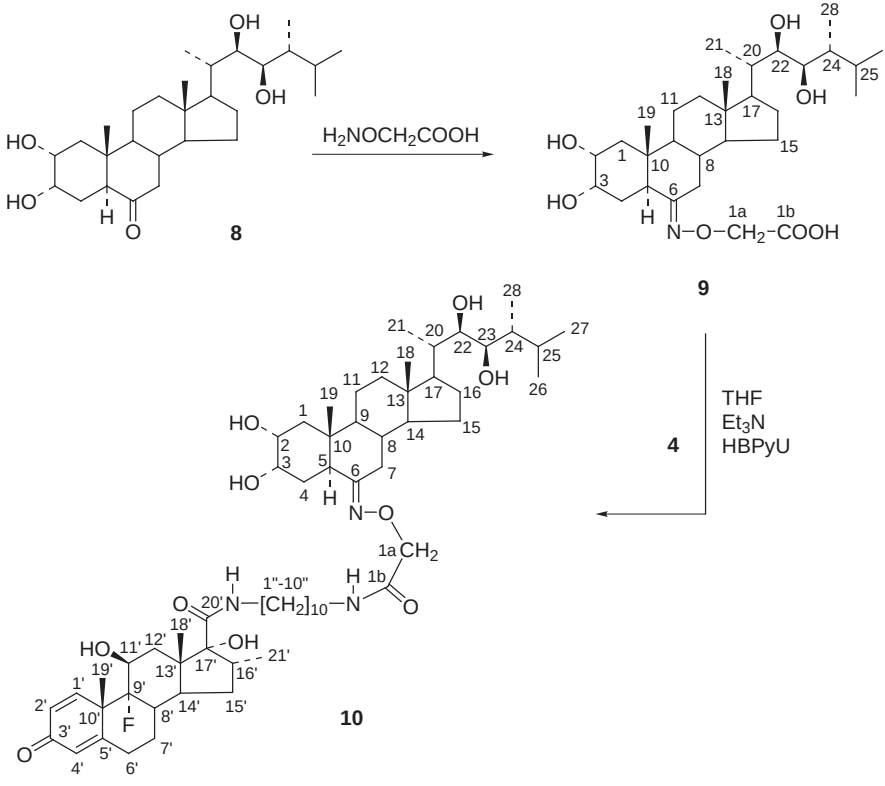
Because of the absence of a carboxylic function in the brassinosteroid 24-epicastasterone (**8**), a different coupling procedure *via* the 6-oxo function was used in this case. Thus, 24-epicastasterone-*O*-(carboxymethyl)oxime (**9**) was synthesised by the reaction of 24-epicastasterone (**8**) with *O*-(carboxymethyl)hydroxylamine hemihydrochloride in 0.1 M ethanolic NaOH in a procedure similar to that described in the ecdysone series[10]. In the next step, oxime **9** was coupled with amine **4** using HBPyU (refs[7,8]). After purification by column chromatography, 58% of amorphous amide **10** was obtained (Scheme 3).

The spectral data of the new compounds are in agreement with the given structures. ^{13}C and relevant ^{1}H NMR data of **2**, **7**, **9** and **10** from one- and two-dimensional NMR experiments including ^{1}H-^{13}C shift correlated spectra (HSQC, HMBC) are shown in Tables I–IV. The unequivocal assignment of ^{1}H and ^{13}C NMR signals in the dexamethasone part and the GA_3 and



SCHEME 2
Synthesis of *N*-[10-(9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamido)decyl]gibberellamide (**7**)

24-epicastasterone moieties in **7** and **10** was made by means of one- and two-dimensional NMR experiments including COSY, NOESY, HSQC, and HMBC techniques. The NMR signals of the methylene chain could not be assigned. The ^{13}C signal at δ 175.4 (**7**) and δ 173.3 (**10**), respectively, was unambiguously assigned to C-20′ by the HMBC correlation with H-16′β. The HMBC correlation between the methylene protons at δ 3.2 and C-20′ proved the coupling of decane-1,10-diamine with C-20′. In a similar manner, the HMBC correlations H-5/C-7, H-6/C-7, and CH_2/C-7 confirmed the coupling of the gibberellin moiety with the decane-1,10-diamine spacer. In the case of **10**, the coupling of the spacer with *O*-(carboxymethyl)oxime **9** was verified by HMBC correlations between H-1a/C-1b and CH_2/C-1b.



SCHEME 3

Synthesis of 6-[({*N*-[10-(9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamido)decyl]carbamoyl}methoxy)imino]-24-epicastasterone (**10**)

TABLE I

^{1}H NMR data (δ, multiplicity, coupling constants [Hz]) for protons of compounds **2** and **7** (the values without multiplicities are chemical shifts of HSQC correlation peaks)

Position	**2**	Position	**7**	Position	**7**
1′	7.415 d [10.1]	1	6.371 dd [9.4/0.6]	1′	7.418 d [10.1]
2′	6.282 dd [10.1/1.9]	2	5.866 dd [9.3/3.5]	2′	6.279 dd [10.2/2.0]
3′	–	3	3.980 d [3.7]	3′	–
4′	6.077 dd [1.9/1.5]	4	–	4′	6.074 br s
5′	–	5	3.256 d [10.1]	5′	–
6′	α: 2.390 ddd [13.6/5.1/1.7] β: 2.718 tdd [13.7/6.2/1.6]	6	2.608 d [10.4]	6′	α: 2.38 β: 2.719 td [13.1/6.1]
7′	α: 1.517 m β: 1.885 dt [13.0/5.3]	7	–	7′	α: 1.51 m β: 1.88 m
8′	2.444 dtd [29.0/12.0/4.9]	8	–	8′	2.44
9′	–	9	1.96	9′	–
10′	–	10	–	10′	–
11′	4.242 ddd [10.9/4.0/2.1]	11	1.70/1.83	11′	4.230 m
12′	α: 2.12; β: 1.592 m	12	1.76/1.97	12′	α: 2.19; β: 1.43
13′	–	13	–	13′	–
14′	2.14	14	1.64/1.90	14′	2.18
15′	α: 1.201 ddd [12.2/8.3/4.2] β: 1.743 dddd [14.2/12.2/11.0/1.5]	15	2.21/2.21	15′	α: 1.19 β:1.74 m
16′	2.996 dqd [11.2/7.2/4.2]	16	–	16′	3.10
17′	–	17	5.177/4.915	17′	–
18′	1.137	18	1.203 s	18′	1.089 s
19′	1.591	19	–	19′	1.589 s
20′	–	1″	*ca* 3.2	20′	–
21′	0.940 d [7.2]	2″–9″	1.30–1.50	21′	0.885 d [7.3]
		10″	*ca* 3.2		

TABLE II

^{1}H NMR data (δ, multiplicity, coupling constants [Hz]) for protons of compounds **9** and **10** (the values without multiplicities are chemical shifts of HSQC correlation peaks)

Position	**9**	Position	**10**	Position	**10**
1	α: 1.45; β: 1.66	1	α: 1.43; β: 1.74	1′	7.301 d [10.0]
2	3.659 d [1.5]	2	3.70	2′	6.321 dd [10.1/1.8]
3	3.928 d [2.4]	3	3.98	3′	–
4	α: 1.797; β: 1.709	4	α: 1.85; β: 1.68	4′	6.112
5	α: 2.401 dd [12.3/3.4]	5	α: 2.41	5′	–
6	–	6	–	6′	α: 2.36; β: 2.62
7	α: 1.29; β: 3.32	7	α: 1.30; β: 3.21	7′	α: 1.53; β: 1.85
8	1.59	8	1.50	8′	2.38
9	1.10	9	1.09	9′	–
10	–	10	–	10′	–
11	α: 1.66; β: 1.33	11	α: 1.61; α: 1.28	11′	4.27
12	α: 1.25; β: 2.03	12	α: 1.24; β: 2.01	12′	α: 2.18; β: 1.39
13	–	13	–	13′	–
14	1.24	14	1.22	14′	2.11
15	1.19/1.67	15	1.15/1.64	15′	α: 1.20; β: 1.75
16	1.30/2.02	16	1.30/2.00	16′	3.10
17	1.57	17	1.56	17′	–
18	0.713	18	0.677	18′	1.098
19	0.768	19	0.736	19′	1.556
20	1.49	20	1.45	20′	–
21	0.972 d [6.7]	21	0.965 d [6.7]	21′	0.918 d [5.3]
22	3.65	22	3.67	1″	3.27/3.16
23	3.30	23	3.36	2″–9″	*ca* 1.5 (8 H) *ca* 1.3 (8 H)
24	1.48	24	1.48	10″	3.28
25	1.94	25	1.92		
26	0.913 d [7.0]	26	0.862 d [6.7]		
27	0.856 d [6.7]	27	0.915 d [7.0]		
28	0.832 d [7.0]	28	0.836 d [6.7]		
1a	4.500/4.503	1a	4.42		
1b	–	1b	–		

TABLE III
^{13}C NMR chemical shifts and coupling constants J_{CF} [Hz] for carbons of compounds **2** and **7**

Position	**2**	Position	**7**	Position	**7**
1′	156.2	1	133.2	1′	156.1
2′	129.8	2	134.1	2′	129.7
3′	189.2	3	70.7	3′	189.0
4′	125.1	4	55.1	4′	125.0
5′	171.3	5	54.3	5′	171.2
6′	32.2	6	53.6	6′	32.3
7′	28.8	7	173.5	7′	28.8
8′	35.7 [19.0]	8	51.7	8′	35.8 [20.1]
9′	102.5 [175.7]	9	52.1	9′	102.6 [175.9]
10′	50.3 [22.5]	10	92.8	10′	50.3 [22.6]
11′	73.1 [37.4]	11	18.1	11′	73.1 [37.7]
12′	36.9	12	39.8	12′	36.9
13′	49.2	13	78.8	13′	49.4
14′	44.3	14	46.1	14′	44.8
15′	33.5	15	44.6	15′	33.4
16′	37.2	16	158.2	16′	36.3
17′	87.8	17	107.2	17′	88.1
18′	17.7	18	14.9	18′	17.8
19′	23.6 [5.8]	19	181.3	19′	23.6 [6.3]
20′	177.0	1″	40.4	20′	175.4
21′	15.3	2″–9″	28.0/28.1/30.3/30.4 30.5/30.6/30.6/30.8	21′	15.2
		10″	40.4		

TABLE IV
^{13}C NMR chemical shifts and coupling constants J_{CF} [Hz] for carbons of compounds **9** and **10**

Position	**9**	Position	**10**	Position	**10**
1	40.7	1	39.2	1′	153.5
2	69.4	2	68.1	2′	129.2
3	69.9	3	68.3	3′	187.3
4	29.2	4	27.3	4′	124.6
5	44.6	5	43.3	5′	167.8
6	174.4	6	162.6	6′	31.0
7	31.7	7	30.4	7′	27.4
8	36.8	8	35.6	8′	34.2 [19.6]
9	55.6	9	53.9	9′	100.9 [175.9]
10	41.4	10	39.5	10′	49.9 [21.1]
11	22.3	11	21.0	11′	71.7 [37.7]
12	41.1	12	39.4	12′	35.7
13	43.9	13	42.6	13′	49.2
14	57.9	14	56.2	14′	43.6
15	25.0	15	23.9	15′	32.1
16	28.9	16	27.5	16′	35.1
17	54.2	17	52.5	17′	86.7
18	12.3	18	11.8	18′	17.0
19	13.3	19	12.8	19′	22.7 [5.3]
20	41.7	20	40.2	20′	173.3
21	13.1	21	12.2	21′	14.3
22	73.5	22	72.3	1″	39.19
23	77.4	23	75.8	2″–9″	29.29
24	42.7	24	41.4	2″–9″	29.24
25	28.0	25	26.8	2″–9″	29.22
26	22.6	26	17.0	2″–9″	29.16
27	17.6	27	22.0	2″–9″	29.00
28	11.2	28	10.6	2″–9″	29.00
1a	70.8	1a	72.1	2″–9″	26.68
1b	162.9	1b	171.0	2″–9″	26.55
				10″	38.62

In conclusion, we have prepared the first heterodimer hybrid molecules of phytohormones gibberellin A_3 and 24-epicastasterone with dexamethasone as the conjugated moiety, which could be used as probes for molecular biological studies of protein–protein interactions, signal transduction pathways and ligand–protein receptor interactions.

EXPERIMENTAL

Materials and Methods

Merck silica gel 60 (particle size 0.040–0.063 mm, 230–400 mesh ASTM) was used for column chromatography. Melting points (uncorrected) were determined on a Boetius heating table. High-resolution MS were measured on an API Qstar/pulsar hybrid quadrupole TOF MS instrument (Applied Biosystems). EIMS (DIS): 70 eV, AMD 402 (AMD Intectra). The positive-ion electrospray (ESI) mass spectra were obtained on a Finnigan MAT TSQ 7000 instrument (electrospray voltage 4.5 kV, heated capillary temperature 220 °C, sheath gas nitrogen) coupled with a Micro-Tech Ultra-Plus MicroLC system equipped with an RP 18-column (5 μm, 1 × 100 mm, Ultrasep). For HPLC, a gradient system was used starting from H_2O : CH_3CN 1 : 1 (each containing 0.2% HOAc) to 1 : 9 within 15 min followed by a 5-min isocratic period; flow rate 70 μl min^{-1}. IR spectra (ν_{max}, cm^{-1}) were obtained on a Bruker IFS 25. UV spectra were measured with a Perkin–Elmer Lambda 16. NMR spectra 1D: Varian Gemini 300 spectrometer, 300.24 and 75.5 MHz, solvents CD_3OD and $CDCl_3$; 2D: (^{1}H-^{1}H-COSY, NOESY, GHMBC, and GHSQC) Varian Unity 500 spectrometer, 499.83 MHz, solvents CD_3OD and $CDCl_3$, TMS internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz.

9α-Fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic Acid (**2**)

9α-Fluoro-16α-methylprednisolone (dexamethasone) (**1**; 2 g, 5.1 mmol) was dissolved in ethanol (400 ml). To the stirred solution, periodic acid (1.37 g, 6 mmol) in water (240 ml) and sulfuric acid (1.53 ml of concentrated H_2SO_4 in 156 ml of distilled water) were added. After stirring for 24 h at room temperature, ethanol was removed under reduced pressure. The crude product was collected by filtration, washed with water and dried in a desiccator with phosphorus pentoxide. The crude acid was recrystallized to yield the product **2** (1.82 g, 94.3%), m.p. 287 °C decomp. (MeOH–H_2O). EIMS, *m/z* (% rel. int.): 378 (M^+, 2), 358 ($[M - HF]^+$, 26), 160 (14), 147 (15), 135 (18), 122 (100), 120 (56), 107 (10), 91 (17). For ^{1}H and ^{13}C NMR data see Tables I and III.

Succinimidyl-9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylate (**3**)

Carboxylic acid **2** (1.7 g, 4.5 mmol) and *N*-hydroxysuccinimide (517 mg, 4.5 mmol) were dissolved in a mixture of dry THF (36 ml) and dry dioxane (36 ml). A solution of dicyclohexylcarbodiimide (927 mg, 4.5 mmol) in dry THF (9 ml) and dry dioxane (9 ml) was added, and the reaction mixture was stirred at room temperature overnight. Dicyclohexylurea was removed by filtration, and the filtrate was concentrated under reduced pressure to yield the title compound (2.1 g, 98.3%), m.p. 254–256 °C decomp. (EtOAc), TLC

(silufol) R_F 0.44 ($CHCl_3$–MeOH 9 : 1, v/v). EIMS, *m/z* (% rel. int.): 475 (M^+, 0.2), 455 ([M – HF]$^+$, 15), 340 (6), 312 (12), 237 (8), 223 (8), 160 (15), 147 (17), 135 (15), 122 (100), 115 (15), 107 (19), 91 (17), 87 (24). LC-ESIMS: retention time 3.30 min; *m/z* 476 ([M + H]$^+$). Analytical HPLC: Hewlett–Packard 1100, LiChrospher 100 RP 18, 5 μm, 125 × 4 mm, MeCN–H_2O (35 : 65), 1 ml min^{-1}, 254 nm, retention time 16.83 min.

N-(10-Aminodecyl)-9α-fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamide (**4**)

To a stirred solution of decane-1,10-diamine (1.58 g, 9.2 mmol) in EtOAc (500 ml), succinimidyl ester **3** (1.45 g, 3.05 mmol) in EtOAc (75 ml) was added under nitrogen atmosphere and the reaction mixture was stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$–MeOH–Et_3N 3 : 1 : 0.04, v/v/v) to yield amorphous product **4** (982 mg, 60.5%). LC-ESIMS: retention time 2.82 min; *m/z* 533 ([M + H]$^+$). Analytical HPLC: Hewlett–Packard 1100, LiChrocart 100 RP 18, 5 μm, 125 × 4 mm, MeCN–0.1% Et_3N (7 : 3), 1 ml min^{-1}, 254 nm, retention time 12.46 min.

Succinimidyl Gibberellate (**6**)

GA_3 (**5**; 1.03 g, 3 mmol) and *N*-hydroxysuccinimide (347 mg, 3 mmol) were dissolved in a mixture of dry THF (15 ml) and dry dioxane (15 ml). Then, at –10 °C, a solution of DCC (638 mg, 3.1 mmol) in dry THF (5 ml) and dry dioxane (5 ml) was added. The reaction mixture was stirred at –10 °C for 2 h, at –5 °C for 16 h, and at room temperature for 5 h. After filtration of precipitated dicyclohexylurea, the solvent was evaporated to yield 1.3 g of product **6** (98.5%), m.p. 205–210 °C (EtOAc), R_F 0.22 ($CHCl_3$–MeOH 9 : 1, v/v). EIMS, *m/z* (% rel. int.): 443 (M^+, 5), 425 (8), 329 (25), 310 (9), 301 (11), 283 (10), 267 (23), 255 (15), 238 (43), 209 (21), 195 (12), 136 (36), 121 (34), 107 (19), 91 (24), 88 (96), 77 (16), 69 (15), 58 (100).

N-[10-(9α-Fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamido)decyl]gibberellamide (**7**)

Method A. To a stirred solution of compound **4** (402 mg, 0.76 mmol) in EtOAc (70 ml) and triethylamine (2 ml), succinimidyl ester **6** (356 mg, 0.8 mmol) dissolved in EtOAc (30 ml) was added. The reaction mixture was stirred at room temperature for 24 h. After addition of ethanol (15 ml) to dissolve the precipitate, the reaction mixture was stirred at room temperature for another 48 h. The organic solvent was evaporated and the residue was purified by column chromatography ($CHCl_3$–MeOH 95 : 5, v/v) to yield the crude product (184 mg), which was rechromatographed (EtOAc–$CHCl_3$ 4 : 1, v/v) to yield 111 mg (16%) of amorphous conjugate **7**. IR (KBr): 3 421 (NH); 2 929 (CH); 2 855 (CH); 1 760 (CO); 1 661 (CO); 1 525, 1 455, 891. UV (MeOH), λ_{max}, nm (ε, dm^3 mol^{-1} cm^{-1}): 206 (18 000), 239 (15 800). EIMS, *m/z* (% rel. int.): 798 ([M – CO_2 – H_2O]$^+$, 2), 778 (9), 760 (19), 523 (71), 466 (35), 439 (55), 277 (73), 248 (100), 239 (70), 222 (75), 199 (49), 156 (61), 121 (72), 69 (45), 55 (78). LC-ESIMS: retention time 3.87 min; *m/z* 861 ([M + H]$^+$). ESI-TOF MS, *m/z*: 861.4870, calculated for $C_{50}H_{70}FN_2O_9$ 861.5060. Analytical HPLC: Hewlett–Packard 1100, LiChrospher 100 RP 18, 5 μm, 250 × 4 mm, MeOH–0.2% HOAc (7 : 3), 1 ml min^{-1}, 254 nm, retention time 11.93 min. For ^{1}H and ^{13}C NMR data, see Tables I and III.

Method B. To a stirred solution of GA_3 (**5**; 34.2 mg, 0.1 mmol) and compound **4** (53.2 mg, 0.1 mmol) in THF (3 ml), HBPyU (43.13 mg, 0.1 mmol) and Et_3N (27.8 µl) was added. The reaction mixture was stirred at room temperature for 45 min and the solvent was evaporated. The residue was purified by column chromatography ($CHCl_3$–MeOH 95 : 5, v/v) to give a white product, which was rechromatographed (EtOAc–$CHCl_3$ 4 : 1, v/v) to yield 48.4 mg (56.9%) of amorphous conjugate **7**. The spectral data were consistent with the data for this compound obtained by *method A*.

24-Epicastasterone-*O*-(carboxymethyl)oxime (**9**)

O-(Carboxymethyl)hydroxylamine hemihydrochloride (275.3 mg, 1.26 mmol) was added to a stirred solution of 24-epicastasterone (**8**; 464 mg, 1 mmol) in 0.1 M ethanolic NaOH (17 ml). The solution was stirred at 90 °C for 3 h and then at room temperature for 16 h. Ethanol was removed under reduced pressure and the residue was dissolved in water. The solution was acidified with hydrochloric acid and extracted with EtOAc. The organic phase was washed several times with water, dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography ($CHCl_3$–MeOH–HOAc 9 : 1 : 0.1, v/v/v) and recrystallized from EtOAc to give 527 mg of **9** (compound **9** was prepared *via* modified procedure by B. Voigt (personal communication)), m.p. 173–174 °C. EIMS, *m/z* (% rel. int.): 537 (M^+, 0.2), 463 (7), 448 (20), 364 (100), 345 (54), 327 (37), 287 (20), 263 (13), 245 (14), 189 (12), 173 (20), 151 (27), 135 (24), 120 (58), 107 (60), 95 (50), 81 (63), 71 (55). For ^1H and ^{13}C NMR data see Tables II and IV.

6-[({*N*-[10-(9α-Fluoro-11β,17α-dihydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxamido)decyl]carbamoyl}methoxy)imino]-24-epicastasterone (**10**)

To a stirred solution of compound **9** (489 mg, 0.91 mmol) and carboxamide **4** (480 mg, 0.9 mmol) in THF (5 ml), HBPyU (392 mg, 0.91 mmol) and Et_3N (276 µl) were added. The reaction mixture was stirred at room temperature for 1 h, and THF was evaporated. The residue was purified by column chromatography ($CHCl_3$–MeOH 95 : 5, v/v) to give (555 mg, 58%) of amorphous conjugate **10**. IR (KBr): 3 426 (NH); 2 932 (CH); 2 868 (CH); 1 664 with long-wave shoulder (CO); 1 531, 1 455, 1 066, 754. UV (MeOH), λ_{max}, nm (ε, $dm^3\ mol^{-1}\ cm^{-1}$): 206 (18 000), 239 (18 200). LC-ESIMS: retention time 14.92 min; *m/z* 1 052 ($[M + 1]^+$). ESI-TOF MS, *m/z*: 1 052.7103, calculated for $C_{61}H_{99}FN_3O_{10}$ 1 052.7309. Analytical HPLC: Hewlett–Packard 1100, LiChrospher 100 RP 18, 5 µm, 250 × 4 mm, MeCN–0.2% HOAc (8 : 2), 1 ml min^{-1}, 254 nm, retention time 25.21 min. For ^1H and ^{13}C NMR data see Tables II and IV.

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REFERENCES

1. Spencer D. M.: *Trends Genet.* **1996**, *12*, 181.
2. Licitra E. J., Liu J. O.: *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 12817.

3. Amara J. F., Clackson T., Rivera V. M., Guo T., Keenan T., Natesan S., Pollock R., Yang W., Courage N. L., Holt D. A., Gilman M.: *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10618.
4. Takahashi N., Phinney B. O., Mac Millan J. (Eds): *Gibberellins*. Springer, Berlin 1991.
5. Adam G., Schmidt J., Schneider B.: P*rog. Chem. Org. Nat. Prod.* **1999**, *78*, 1.
6. Govindan M. V., Manz B.: *Eur. J. Biochem.* **1980**, *108*, 47.
7. Manz B., Heubner A., Köhler I., Grill H. J., Pollow K.: *Eur. J. Biochem.* **1983**, *131*, 333.
8. Chen S., Xu J.: *Tetrahedron Lett.* **1992**, *33*, 647.
9. Knorr R., Trzeciak A., Bannwarth W., Gillessen D.: *Tetrahedron Lett.* **1989**, *30*, 1927.
10. Beckers C., Emmerich H.: *Naturwissenschaften* **1973**, *60*, 50.