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# Synthesis of new steroidal derivatives by the reaction of steroid–amino acid conjugates with *N*,*N*′-dicyclohexyl-carbodiimide. Unusual formation of steroidal imide derivatives

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ABSTRACT

Several new steroid derivatives have been synthesised by the reaction of steroid–amino acid conjugates with *N*,*N*′-dicyclohexyl-carbodiimide. Selectivity of the reaction was found to depend greatly on the properties of the amino acid moiety. While the reaction of the glycine derivative led to the corresponding 5(4*H*)-oxazolone, an unusual imide formation together with an *N*-acylurea side product was observed in the case of conjugates of L-alanine, L-phenylalanine and L-methionine. The structures of the new products, steroidal imide and *N*-acylurea derivatives, were determined by various spectroscopic methods.



## 1. Introduction

Several steroids with heterocylic moieties (e.g., imidazolyl, pyridyl, pyrazolyl, isoxazolyl, thiazolyl and oxazolyl) on C-17 show interesting biological properties and can be used as inhibitors of 17α-hydroxylase-$C_{17,20}$-lyase[1] but until now there are no examples of the syntheses of steroidal 5(4*H*)-oxazolone derivatives.

5(4*H*)-Oxazolones[2] are known to be produced easily from *N*-acyl amino acid derivatives with dehydrating agents like activated anhydrides[3] or carbodiimides.[4] At the same time the oxazolone ring system contains numerous reactive sites and undergoes a number of reactions such as acylation, alkylation, arylation, etc.[5] In addition, they can be used for the synthesis of oxazoles, β-lactams, pyrroles, pyrrolines, imidazoles and imidazolines, so they also serve as useful intermediates.[5]

Recently, we reported a high-yielding synthesis of new steroid amino acid ester conjugates via palladium-catalysed aminocarbonylation of 17-iodo-5α-androst-16-ene in the presence of amino acid esters as nucleophiles.[6] Based on these studies we decided to investigate the possibility of the synthesis of new steroidal 5(4*H*)-oxazolone derivatives starting from the hydrolysis products of esters **1a**–**4a** using *N*,*N*′-dicyclohexyl-carbodiimide (DCC) as the activating agent. This method had been used successfully by Beck and co-workers for the synthesis of various ferrocenyl-oxazolones.[4]

## 2. Results and discussion

The starting steroid–amino acid conjugates (**1a**–**4a**) were obtained as described previously,[6] by palladium-catalysed aminocarbonylation of 17-iodo-5α-androst-16-ene using amino acid esters as *N*-nucleophiles. Hydrolysis of methyl esters **1a**–**4a** under basic conditions led to the carboxylic acid products **1b**–**4b** in high yields (Scheme 1).

Carboxylic acids **1b**–**4b** were reacted with DCC under the conditions reported by Beck and co-workers for the synthesis of ferrocenyl-oxazolones.[4] However, only reaction of **1b** with DCC resulted in the formation of the expected 5(4*H*)-oxazolone derivative **1c** in good yield (Scheme 2, Table 1, entry 1). It should be noted that **1c** is very sensitive to hydrolysis in solution and decomposes giving **1b** after a few days.

At the same time, only traces of compounds of similar structure **2c** and **4c** could be detected by GC–MS[7] in the reaction mixtures of DCC and acids **2b** and **4b**, respectively, and no formation of an oxazolone derivative was observed starting from **3b**. Instead, two compounds of very different structure were isolated from these reaction mixtures that were proved to be imides **2d**–**4d** and *N*-acylurea derivatives **2e**–**4e** (Scheme 2, entries 2–4).

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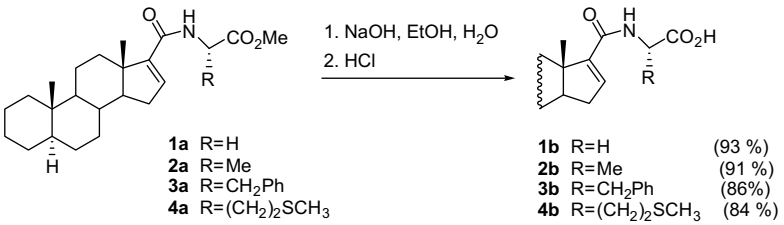
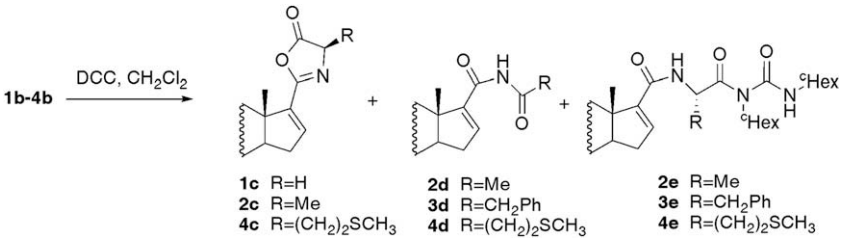



**Scheme 1.** Synthesis of steroid–amino acid conjugates **1b**–**4b**.




**Scheme 2.** Reaction of steroid–amino acid conjugates **1b**–**4b** with DCC.

Formation of the latter compounds is not surprising as carboxylic acids are known to react with carbodiimides such as DCC to give *N*-acylureas via an *O*-acyl→*N*-acyl rearrangement reaction of the *O*-acylisourea type active esters.[8] Although the high reactivity of the *O*-acylisourea intermediates is reported to provoke racemisation in some cases, the *N*-acylurea derivatives **2e**–**4e** were isolated as single isomers.

However, the formation of imides was completely unexpected. Although unsymmetrical imides have been produced by oxidative decarboxylation of *N*-acyl amino acids[9] induced by $Ag^{+}/Cu^{2+}/S_2O_8^{2-}$, to the best of our knowledge, no imide derivatives were isolated from reaction mixtures of amino acid derivatives and DCC. Our experiments show that in some cases, the possibility of the formation of imide-type side products should be taken into consideration.[10]

**Table 1**
The effect of the reaction conditions on product distribution of reactions of **1b**–**4b** with DCC

| Entry | Substrate | Method[a] | Yields of steroid derivatives (%) | | | |
|---|---|---|---|---|---|---|
| | | | **c** | **d** | **e** | **b**[b] |
| 1 | **1b** | A | 91 | — | — | 2 |
| 2 | **2b** | A | —[c] | 35 | 36 | 5 |
| 3 | **3b** | A | — | 61 | 26 | 5 |
| 4 | **4b** | A | —[c] | 42 | 35 | 6 |
| 5 | **2b** | B | — | 28 | 40 | 11 |
| 6 | **3b** | B | — | 30 | 42 | 12 |
| 7 | **2b** | C | — | 9 | 10 | 70 |
| 8 | **3b** | C | — | 16 | 13 | 65 |
| 9 | **4b** | C | — | 17 | 8 | 67 |
| 10 | **2b** | D | —[c] | 9 | 68 | 3 |
| 11 | **4b** | D | —[c] | 7 | 72 | 5 |
| 12 | **2b** | E | — | — | 32 | 62 |
| 13 | **3b** | E | — | — | 43 | 51 |
| 14 | **4b** | E | — | — | 37 | 56 |
| 15 | **2b** | F | — | 42 | 38 | 4 |
| 16 | **2b** | G | — | 15 | 25 | 51 |
| 17 | **3b** | G | — | 17 | 26 | 56 |
| 18 | **4b** | G | — | 11 | 23 | 54 |

[a] DCC was added dropwise at 0 °C in $CH_2Cl_2$ to the solution of the substrate over 1 h. Method A: after addition of DCC, the mixture was stirred for 7 h at rt. Method B: after addition of DCC, the mixture was stirred for 7 h at 40 °C. Method C: after addition of DCC, the mixture was stirred for 7 h at 0 °C. Method D: the reaction was carried out according to method A by protecting from light. Method E: the reaction was carried out according to method A in an inert atmosphere. Method F: the reaction was carried out according to method A but oxygen was bubbled through the reaction mixture. Method G: the reaction was carried out according to method A in the presence of Pd/C (10 w/w% Pd) (**2b**–**4b**/DCC/Pd=1:1:0.05).

[b] Substrate recovered.

[c] The presence of the corresponding 5(4*H*)-oxazolone could be detected by GC–MS in the reaction mixture.[7]

At the same time, unsymmetrical acyclic imides represent a very important group of compounds as they usually show high biological (e.g., antimicrobial or antifungal) activity. This structural motif also appears in certain natural products such as *fumaramidmycin*[11] or *coniothyriomycin*.[12] In the past two decades, design of bioconjugates consisting of two entities having different biological activities has been receiving increasing attention. Among the hybrid natural products, hybrids of steroid frameworks have attracted great attention due to the significant biological properties and numerous therapeutic effects of steroids.[13] The reaction of steroidal amino acids with DCC leading to steroidal imides presented here can be an important addition to the already known synthetic methods of producing steroid conjugates.

In order to obtain a better insight into the reaction, the distribution of products **2d**–**4d**/**2e**–**4e** was investigated using various reaction conditions (Table 1).

Temperature is known to have a decisive effect on the outcome of the reaction: at lower temperatures the formation of *O*-acylisourea and oxazolone derivatives is favoured, while an increase in the temperature usually leads to *N*-acylurea-type products in higher yields.[8b,d] In our case, stirring the reaction mixtures at 40 °C instead of rt resulted in a slight decrease in the amount of the imide derivatives **2d** and **3d**, and an increase in the yield of *N*-acylurea products **2e** and **3e** (Table 1, entries 5 and 6). No formation of oxazolones could be detected. Lowering the temperature resulted only in a decrease in the reaction rate and an increase in the amount of recovered starting material (entries 7–9).

It was proved that product distribution was greatly influenced by both light and air. The effect of light was demonstrated by carrying out the reactions of **2b** and **4b** while protecting the reaction mixture from light (Table 1, entries 10 and 11). The amount of the steroidal imide derivatives **2d** and **4d** was decreased dramatically, while the yield of **2e** and **4e** increased. At the same time, the presence of 5(4*H*)-oxazolones **2c** and **4c** was detected again by GC–MS. The use of inert atmosphere prevented imide formation completely. At the same time, it led to an increase in the amount of the recovered starting material, thus lowering the reaction rate. On the contrary, when oxygen was bubbled through the reaction mixture,

a slight increase in the yield of the imide derivative **2d** was observed (compare entries 2 and 15).

Under UV irradiation and in air 5(4*H*)-oxazolones were reported to go through oxidative photodecarboxylation and to decompose to unsymmetrical acyclic imides through an endoperoxide intermediate.[14] Although a certain explanation for the formation of steroidal imide derivatives **2d**–**4d** under our conditions cannot be given, it is possible that a similar reaction takes place (Scheme 3). This is supported by the fact that both the use of inert atmosphere and protection of the reaction mixtures from light led to a marked decrease in the yields of imide products.

Although *N*-acylureas are known to be unreactive, the possibility of transforming **2e**–**4e** to **2d**–**4d** was investigated, too. However, besides the starting material no other steroid derivatives could be detected in the reaction mixtures of **2e**–**4e** and DCC under the same conditions given in entries 1–4, Table 1.

5(4*H*)-Oxazolones were reported to be converted to imides via Pd/C-catalyzed oxidation and decarboxylation by Bates and coworkers,[15] so the reactions of **2b**–**4b** with DCC were carried out in the presence of Pd/C (entries 16–18). Unfortunately, the yield of imides **2d**–**4d** could not be increased even in the presence of the catalyst.

## 3. Conclusions

In conclusion it can be stated that either a new steroidal 5(4*H*)-oxazolone (**1c**) or new steroidal *N*-acylureas (**2e**–**4e**) and imide derivatives (**2d**–**4d**) can be obtained in moderate to good yields by the reaction of steroid–amino acid conjugates with DCC. Although oxazolone **1c** could be produced in excellent yield from the glycine derivative **1b**, the use of other steroidal carboxylic acids (**2b**–**4b**) led to the formation of mixtures of *N*-acylureas **2e**–**4e** and imides **2d**–**4d**. Formation of imide derivatives can be explained by spontaneous oxidative decarboxylation of the primarily formed oxazolone derivatives **2c**–**4c**.

Although oxidative decarboxylation of 5(4*H*)-oxazolones in the presence of oxidising agents or under UV irradiation is a known process, the spontaneous reaction of *N*-acyl amino acids and DCC leading to imides or decarboxylation of oxazolones without the use of any inducers is unprecedented.

The reactivity of *N*-acyl amino acids is known to depend greatly on both the choice of the *N*-acyl group, in our case the steroid moiety, and the type of the amino acid itself. This could be the reason for the unique behaviour of **2b**–**4b**.

## 4. Experimental

### 4.1. General procedures

$^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ on a Varian Inova 400 spectrometer at 400.13 MHz and 100.62 MHz, respectively. Chemical shifts $\delta$ are reported in parts per million relative to $CHCl_3$ (7.26 and 77.00 ppm for $^1$H and $^{13}$C, respectively). Mass spectra of **1c** and **3d** were recorded on an HP-5971A MSD connected to an HP-5890/II gas chromatograph. MS measurements of **1b**, **2d** and **2e** were carried out on a Finnigan MAT95 SQ mass spectrometer using FIB ionisation (Cs ion, glycerine matrix, 20 kV) for compound **1b**, and EI ionisation technique (70 eV, 220 °C source temperature) for compounds **2d** and **2e**. High-resolution MS measurements of **2b**, **3b**, **4b**, **4d**, **3e** and **4e** were carried out on a Thermo LTQ FT Ultra mass spectrometer (ESI, 4.0 kV spray voltage, 295 °C capillary temperature, solvent: MeOH/$H_2O$ 1:1+1 v/v % cc. AcOH). The protonated molecular ion peaks were fragmented by CID at a normalised collision energy of 10–14%. The relative abundance values of the fragment ions in the MS–MS spectrum are given in brackets. IR spectra were made using an Avatar 330 FT-IR instrument. Samples were prepared as KBr pellets. Elemental analyses were measured on a 1108 Carlo Erba apparatus.

Methyl esters **1a**–**4a** were obtained as described previously.[6a]

### 4.2. General procedure for the synthesis of steroid–amino acid conjugates (1b–4b)

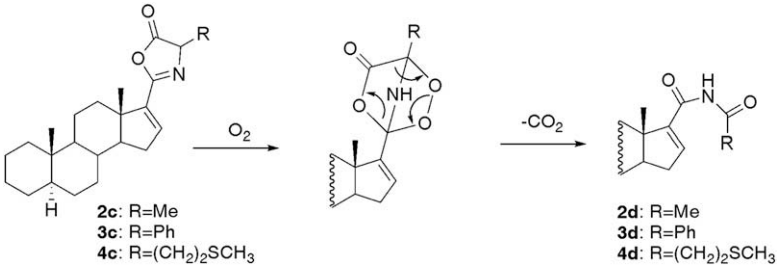
The steroidal amino acid ester (**1a**–**4a**, 0.5 mmol) was dissolved in ethanol (15 mL) and then a slight excess of 0.1 M NaOH (30 mL) was added dropwise at 0 °C. The solution was stirred for 7 h at rt and an equimolar amount of 0.1 M HCl (to the amount of NaOH) was added dropwise. The mixture was extracted with $CH_2Cl_2$ (3×50 mL). The combined organic phases were dried over $Na_2SO_4$ and the solvent was removed by rotary evaporation. The products were purified by column chromatography (silica gel, chloroform/MeOH=8:2).

### 4.3. General procedures for the reactions of 1b–4b with dicyclohexyl-carbodiimide (DCC)

*Method A*. The steroid–amino acid conjugate (**1b**–**4b**, 0.3 mmol) was suspended in $CH_2Cl_2$ (6 mL). At 0 °C a solution of an equimolar amount of DCC (0.3 mmol) in $CH_2Cl_2$ (3 mL) was added dropwise in 1 h. After 7 h stirring at rt, the solvent was removed by rotary evaporation. The products were purified by column chromatography (silica gel, *n*-hexane/EtOAc=8:2).

*Method B*. The steroid–amino acid conjugate (**2b** or **3b**, 0.3 mmol) was suspended in $CH_2Cl_2$ (6 mL). At 0 °C a solution of an equimolar amount of DCC (0.3 mmol) in $CH_2Cl_2$ (3 mL) was added dropwise in 1 h. After 7 h stirring at 40 °C, the solvent was removed by rotary evaporation. The products were purified by column chromatography (silica gel, *n*-hexane/EtOAc=8:2).

*Method C*. The steroid–amino acid conjugate (**2b**–**4b**, 0.3 mmol) was suspended in $CH_2Cl_2$ (6 mL). At 0 °C a solution of an equimolar amount of DCC (0.3 mmol) in $CH_2Cl_2$ (3 mL) was added dropwise in 1 h. After 7 h stirring at 0 °C, the solvent was removed by rotary evaporation. The products were purified by column chromatography (silica gel, *n*-hexane/EtOAc=8:2).




**Scheme 3.** Possible mechanism for the formation of imides **2d**–**4d**.

*Method D*. Same as method A but addition of the DCC solution and stirring at rt was carried out by preventing from light.

*Method E*. Same as method A but addition of the DCC solution and stirring at rt was carried out in an argon atmosphere.

*Method F*. Same as method A but oxygen was bubbled through the mixture throughout the reaction.

*Method G*. The steroid–amino acid conjugate (**2b**–**4b**, 0.3 mmol) and 15 mg Pd/C (10 w/w % Pd) were suspended in $CH_2Cl_2$ (6 mL). At 0 °C a solution of an equimolar amount of DCC (0.3 mmol) in $CH_2Cl_2$ (3 mL) was added dropwise in 1 h. After 7 h stirring at rt, the solvent was removed by rotary evaporation. The products were purified by column chromatography (silica gel, *n*-hexane/EtOAc=8:2).

### 4.4. Characterisation of the products

*4.4.1. N-(17-Androst-16-enoyl)-glycine (**1b**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 6.50 (br s, 1H), 6.42 (br s, 1H), 6.05 (br s, 1H+$H_2O$ (solvent)), 4.10 (br s, 2H), 0.60–2.20 (m, 22H, ring protons), 0.95 (s, 3H), 0.78 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$): 179.2, 166.7, 149.8, 138.1, 57.0, 55.3, 47.5, 46.7, 42.1, 38.7, 36.7, 35.2, 34.0, 32.1, 31.9, 29.2, 29.1, 27.0, 22.4, 20.9, 16.7, 12.4. IR (KBr, $cm^{-1}$): 3391, 1732, 1646, 1587. MS: M+H: *m*/*z* 360, MS–MS of *m*/*z* 360: *m*/*z* 285 (100), 257 (3). Analysis calculated for $C_{22}H_{33}NO_3$ C, 73.50; H, 9.25; N, 3.90. Found: C, 73.64; H, 9.27; N, 3.96. White solid, mp 155–156 °C. Isolated yield: 93%.

*4.4.2. N-(17-Androst-16-enoyl)-alanine (**2b**)*

$\delta_H$ (400.13 MHz, $CDCl_3$/DMSO-$d_6$ 1:1): 7.15–7.22 (m, 1H), 6.22–6.29 (m, 1H), 3.95 (br s, 1H), 3.39 (br s, 1H+$H_2O$ (solvent)), 1.24 (d, *J* 6.7 Hz, 3H), 0.63–2.20 (m, 22H, ring protons), 0.88 (s, 3H), 0.76 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$/DMSO-$d_6$ 1:1): 182.6, 163.7, 150.6, 134.1, 56.3, 54.5, 46.6, 45.8, 45.4, 37.9, 35.9, 34.5, 33.3, 31.4, 30.9, 28.5, 28.4, 26.2, 21.6, 20.2, 19.0, 16.1, 11.8. IR (KBr, $cm^{-1}$): 3417, 1736, 1636, 1589. HRMS M+H: *m*/*z* 374.26846, calculated value for $C_{23}H_{36}NO_3$: 374.26897 (delta: −1.36 ppm). MS–MS of *m*/*z* 374: *m*/*z* 285. Analysis calculated for $C_{23}H_{35}NO_3$: C, 73.96; H, 9.44; N, 3.75. Found: C, 74.18; H, 9.47; N, 3.88. White solid, mp 176–178 °C. Isolated yield: 91%.

*4.4.3. N-(17-Androst-16-enoyl)-phenylalanine (**3b**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 7.00–7.20 (m, 5H), 6.51 (br s, 1H), 6.08–6.13 (m, 1H), 4.45–4.55 (m, 1H), 3.60 (br s, 1H+$H_2O$ (solvent)), 3.18–3.30 (m, 1H), 2.94–3.07 (m, 1H), 0.50–1.96 (m, 22H, ring protons), 0.70 (s, 6H). $\delta_C$ (100.62 MHz, $CDCl_3$): 178.9, 166.5, 149.8, 138.3, 136.9, 129.4, 128.1, 126.1, 56.5, 54.9, 51.9, 47.1, 46.1, 38.4, 37.5, 36.3, 34.6, 33.6, 31.8, 31.5, 29.0, 28.8, 26.7, 22.1, 20.6, 16.3, 12.0. IR (KBr, $cm^{-1}$): 3419, 1708, 1642, 1602. HRMS M+H: *m*/*z* 450.30011, calculated value for $C_{29}H_{40}NO_3$: 450.30027 (delta: −0.36 ppm). MS–MS of *m*/*z* 450: *m*/*z* 432 (5), 404 (3), 285 (100). Analysis calculated for $C_{29}H_{39}NO_3$: C, 77.47; H, 8.74; N, 3.12. Found: C, 77.67; H, 8.80; N, 3.22. White solid, mp 158–160 °C. Isolated yield: 86%.

*4.4.4. N-(17-Androst-16-enoyl)-methionine (**4b**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 7.02 (br s, 1H), 6.43 (br s, 1H), 4.12–4.29 (m, 1H), 2.79 (br s, 1H), 2.39–2.53 (m, 2H), 2.03 (s, 3H), 0.7–2.20 (m, 24H, ring protons+$CH_2CH_2S$), 0.90 (s, 3H), 0.79 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$): 174.7, 166.5, 149.9, 138.4, 57.0, 55.3, 52.1, 47.4, 46.7, 38.7, 36.7, 35.2, 34.0, 32.1, 31.9, 31.7, 30.3, 29.2, 29.1, 27.0, 22.3, 20.9, 16.8, 15.7, 12.4. IR (KBr, $cm^{-1}$): 3396, 1712, 1653, 1593. HRMS M+H: *m*/*z* 434.27187, calculated value for $C_{25}H_{40}NO_3S$: 434.27234 (delta: −1.09 ppm). MS–MS of *m*/*z* 434: *m*/*z* 416 (13), 386 (5), 285 (100). Analysis calculated for $C_{25}H_{39}NO_3S$: C, 69.24; H, 9.06; N, 3.23. Found: C, 69.42; H, 9.11; N, 3.15. White solid, mp 149–150 °C. Isolated yield: 84%.

*4.4.5. 2-(Androst-16-ene-17-yl)-5(4H)-oxazolone (**1c**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 6.61–6.68 (m, 1H), 4.25 (s, 2H), 0.72–2.40 (m, 22H, ring protons), 0.92 (s, 3H), 0.80 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$): 176.0, 160.7, 142.7, 141.8, 56.9, 55.2, 54.8, 47.3, 46.4, 38.4, 36.5, 34.9, 33.9, 32.3, 32.0, 29.0, 28.9, 26.8, 22.1, 20.6, 16.1, 12.1. IR (KBr, $cm^{-1}$): 1826, 1650, 1627. MS *m*/*z* 341 (25) ($M^+$), 326 (8), 284 (31), 269 (28), 257 (9), 178 (14), 148 (10), 121 (24), 105 (35), 91 (54), 67 (87), 55 (100). Analysis calculated for $C_{22}H_{31}NO_2$: C, 77.38; H, 9.15; N, 4.10. Found: C, 77.65; H, 8.95; N, 3.86. White solid, mp 132–134 °C. Isolated yield: 91% (method A).

*4.4.6. 17-(N-Acetyl-carbamoyl)-androst-16-ene (**2d**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 8.16 (br s, 1H), 6.51–6.55 (m, 1H), 2.50 (s, 3H), 0.70–2.40 (m, 22H, ring protons), 0.97 (s, 3H), 0.81 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$): 173.1, 163.5, 150.1, 140.9, 56.7, 55.3, 47.5, 47.2, 38.7, 36.7, 34.8, 34.0, 32.4, 32.1, 29.2, 29.0, 27.0, 25.5, 22.3, 20.8, 16.5, 12.4. IR (KBr, $cm^{-1}$): 3284, 1711, 1689, 1591. MS *m*/*z* (rel int. %): 343 (3) ($M^+$), 328 (12), 284 (100), 269 (87), 257 (24), 241 (5), 161 (6), 147 (7), 121 (9), 105 (8), 91 (8), 43 (10). Analysis calculated for $C_{22}H_{33}NO_2$: C, 76.92; H, 9.68; N, 4.08. Found: C, 76.95; H, 9.47; N, 4.24. White solid, mp 151–153 °C. Isolated yield: 35% (method A).

*4.4.7. 17-(N-(Phenyl-acetyl)-carbamoyl)-androst-16-ene (**3d**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 8.01 (br s, 1H), 7.15–7.40 (m, 5H), 6.45 (s, 1H), 4.18 (s, 2H), 0.80–2.34 (m, 22H, ring protons), 0.96 (s, 3H), 0.81 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$): 172.9, 163.0, 149.9, 140.7, 133.8, 129.8, 128.6, 127.1, 56.5, 55.1, 47.2, 47.0, 43.7, 38.4, 36.5, 34.6, 33.8, 32.2, 31.9, 29.0, 28.8, 26.8, 22.1, 20.6, 16.3, 12.2. IR (KBr, $cm^{-1}$): 3326, 1710, 1638, 1589. MS *m*/*z* 419 (43) ($M^+$), 376 (100), 348 (12), 257 (10), 217 (31), 144 (62), 118 (47), 91 (54), 67 (33). Analysis calculated for $C_{28}H_{37}NO_2$: C, 80.15; H, 8.89; N, 3.34. Found: C, 80.02; H, 8.98; N, 3.51. White solid, mp 144–145 °C. Isolated yield: 61% (method A).

*4.4.8. 17-(N-(3-Methyl-thio-propionyl)-carbamoyl)-androst-16-ene (**4d**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 8.06 (br s, 1H), 6.49–6.51 (m, 1H), 2.80 (t, *J* 7.1 Hz, 2H), 2.13 (s, 3H), 0.80–2.35 (m, 24H ring protons+$CH_2CH_2S$), 0.91 (s, 3H), 0.80 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$): 173.7, 163.1, 149.8, 140.7, 56.4, 55.0, 47.2, 46.9, 38.4, 37.5, 36.4, 34.5, 33.7, 32.2, 31.8, 28.9, 28.8, 28.3, 26.7, 22.0, 20.5, 16.2, 15.6, 12.1. IR (KBr, $cm^{-1}$): 3325, 1711, 1626, 1573. HRMS: M+H: *m*/*z* 404.26113, calculated value for $C_{24}H_{38}NO_2S$: 404.26178 (delta: −1.60 ppm). MS–MS of *m*/*z* 404: *m*/*z* 356 (6), 285 (100), 257 (2). Analysis calculated for $C_{24}H_{37}NO_2S$: C, 71.42; H, 9.24; N, 3.47. Found: C, 71.62; H, 9.01; N, 3.61. White solid, mp 132–134 °C. Isolated yield: 42% (method A).

*4.4.9. N,N′-Dicyclohexyl-N′-(2-(5α-androst-16-ene-17-carboxamido)-propionyl)-urea (**2e**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 7.50–7.62 (m, 1H), 6.39–6.43 (m, 1H), 6.06 (d, *J* 6.7 Hz, 1H), 4.67 (qui, *J* 6.7 Hz, 1H), 4.05–4.15 (m, 1H), 3.60–3.70 (m, 1H), 1.33 (d, *J* 6.7 Hz, 3H), 0.70–2.20 (m, 42H, steroidal ring protons+$^C$Hex ring protons), 0.94 (s, 3H), 0.79 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$): 171.8, 166.4, 153.5, 149.5, 137.9, 56.8, 55.0, 54.8, 50.3, 48.0, 47.2, 46.4, 38.4, 36.4, 35.0, 33.8, 32.6, 31.8, 31.7, 31.6, 31.4, 29.3, 29.0, 28.8, 26.7, 26.0, 25.9, 25.4, 25.3, 24.8 (2C), 22.1, 20.6, 18.5, 16.4, 12.1. IR (KBr, $cm^{-1}$): 3399, 1703, 1646, 1593. MS *m*/*z* 579 (0.04) ($M^+$), 454 (7), 411 (9), 356 (6), 329 (100), 314 (31), 285 (89), 257 (5). Analysis calculated for $C_{36}H_{57}N_3O_3$: C, 74.57; H, 9.91; N, 7.25. Found: C, 74.78; H, 9.72; N, 7.41. White solid, mp 150–151 °C. Isolated yield: 68% (method D).

*4.4.10. N,N′-Dicyclohexyl-N′-(2-(5α-androst-16-ene-17-carboxamido)-3-phenyl-propionyl)-urea (**3e**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 7.36 (d, *J* 7.9 Hz, 1H), 7.00–7.30 (m, 5H), 6.28–6.35 (m, 1H), 5.95–6.05 (m, 1H), 4.82–4.93 (m, 1H), 4.05–4.17 (m, 1H), 3.59–3.73 (m, 1H), 3.10 (dd, *J* 5.7, 13.6 Hz, 1H), 2.90 (dd, *J* 9.5, 13.6 Hz, 1H), 0.70–2.20 (m, 42H, steroidal ring protons+$^C$Hex ring protons), 0.92 (s, 3H), 0.78 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$): 172.1, 166.2, 153.2, 149.4, 138.2, 136.1, 129.1, 128.7, 127.1, 56.7, 55.0, 53.5,

53.3, 50.3, 47.2, 46.2, 38.7, 38.4, 36.4, 34.9, 33.7, 32.5, 31.8, 31.6, 31.5, 31.4, 29.3, 29.0, 28.8, 26.7, 26.0, 25.9, 25.4, 25.3, 24.7, 22.1, 20.6, 16.4, 12.1. IR (KBr, $cm^{-1}$): 3427, 2, 1709, 1638, 1591. HRMS: M+H: *m/z* 656.47708, calculated value for $C_{42}H_{62}N_3O_3$: 656.47857 (delta: −2.27 ppm). MS–MS of *m/z* 656: *m/z* 572. Analysis calculated for $C_{42}H_{61}N_3O_3$: C, 76.90; H, 9.37; N, 6.41. Found: C, 76.78; H, 9.61; N, 6.27. White solid, mp 157–159 °C. Isolated yield: 43% (method E).

#### *4.4.11. N,N′-Dicyclohexyl-N′-(2-(5α-androst-16-ene-17-carboxamido)-4-methyl-thio-butanoyl)-urea (**4e**)*

$\delta_H$ (400.13 MHz, $CDCl_3$): 7.54–7.64 (m, 1H), 6.42–6.50 (m, 1H), 6.22 (d, *J* 7.5 Hz, 1H), 4.78–4.86 (m, 1H), 4.09–4.19 (m, 1H), 3.62–3.71 (m, 1H), 2.51 (t, *J* 6.8 Hz, 2H), 2.08 (s, 3H), 0.72–2.20 (m, 44H, steroidal ring protons+$^c$Hex ring protons+$CH_2CH_2S$), 0.94 (s, 3H), 0.79 (s, 3H). $\delta_C$ (100.62 MHz, $CDCl_3$): 170.4, 166.6, 153.3, 149.4, 138.2, 56.8, 55.0, 54.8, 51.5, 50.3, 47.2, 46.4, 38.4, 36.4, 35.0, 33.8, 32.6, 31.9, 31.8, 31.7, 31.6, 31.5, 30.2, 29.2, 29.0, 28.8, 26.7, 25.9, 25.8, 25.4, 25.3, 24.6 (2C), 22.1, 20.6, 16.4, 15.4, 12.1. IR (KBr, $cm^{-1}$): 3452, 3329, 1706, 1636, 1590. HRMS: M+H: *m/z* 640.44912, calculated value for $C_{38}H_{62}N_3O_3S$: 640.45064 (delta: −2.38 ppm). MS–MS of *m/z* 640: *m/z* 622 (12), 556 (100), 515 (57), 416 (14), 357 (10), 250 (12), 224 (50). Analysis calculated for $C_{38}H_{61}N_3O_3S$: C, 71.32; H, 9.61; N, 6.57. Found: C, 71.61; H, 9.79; N; 6.28. White solid, mp 136–138 °C. Isolated yield: 72% (method D).

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