Novel protective group synthesis of androgen receptor modulators with steroidal and nonsteroidal scaffolds

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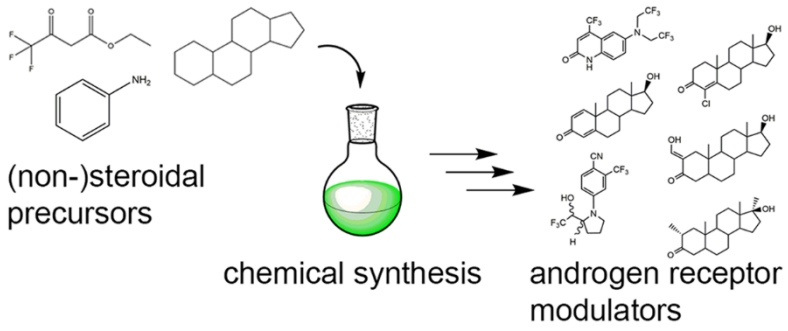


Abstract

Anabolic androgenic steroids (AAS) are frequently used either clinically, by athletes, or for body shaping due to their muscle building and performance enhancing properties. AAS misuse is associated with cardiovascular diseases, mood changes and endocrine issues. Despite the recognition of the severe adverse effects of AAS misuse, the underlying molecular mechanisms are insufficiently understood. Selective androgen receptor modulators (SARMs) are supposed to diminish the adverse androgenic AAS effects while maximizing anabolic effects. In order to obtain androgen receptor modulating compounds of high purity for mechanistic in vitro investigations, this study summarizes protocols of optimized chemical synthesis for five AAS and two SARMs.

The procedures described exhibit the following advantages:

1. straightforward, easily reproducible synthesis method

2. high purity achieved

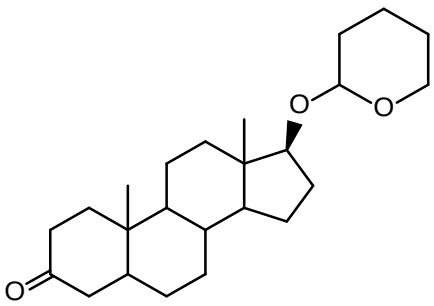
3. low cost chemical synthesis

Keywords

steroid synthesis; anabolic androgenic steroids; selective androgen receptor modulators

Method details

Synthesis of drostanolone:



STEP I: Androstan-17β-((tetrahydro-2H-pyran-2-yl)oxy)-3-one (1)

Facilitating higher yields, the application of the tetrahydropyranyl-(THP)-group in order to protect the 17β-alcohol as a THP-ether was established:

3.53 g Dihydropyran was added dropwise to a solution of 10.2 g dihydrotestosterone in 200 ml dichloromethane under argon at room temperature, followed by the addition of a solution of 0.100 g pyridinium tosylate in 13 ml dichloromethane.

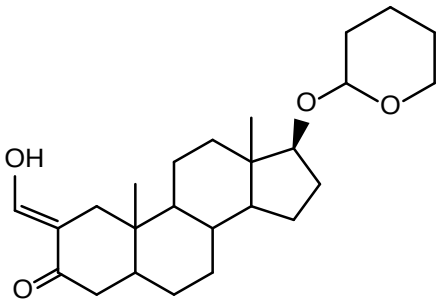
After 20 h stirring at room temperature, TLC showed 80% conversion. An additional amount of 0.420 g dihydropyran and 0.010 g pyridinium tosylate were added to the solution and stirring continued for another 24 h. After this period, a total conversion of approx. 90% could be monitored by TLC.

The reaction mixture was diluted with 200 ml dichloromethane and then washed with water and saturated sodium chloride solution. The combined organic extracts were dried over $MgSO_4$ and evaporated to yield 16.50 g of colorless foam.

The crude product was chromatographed on silica gel by using hexane-ethyl acetate (1:1) as an eluent. The isolated product was obtained as a colorless solid (13.10 g; **~ 99%**).

MS (ESI^+):
m/z = 374.89 (calculated MW: 374.56 g/mol)

R_f: 0.7, hexane/ethyl acetate 1:1, visualized by Seebach derivatization reagent.



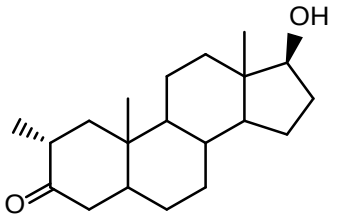
STEP II: 2-Hydroxymethylene-androstan-17β-((tetrahydro-2H-pyran-2-yl)oxy)-3-one (2)

13.00 g Intermediate (1) was completely dissolved in 200 ml dry tetrahydrofuran. 1.09 g Sodium hydride was added in small portions before the reaction mixture was flushed with argon and stirred at room temperature for 1 h. After this period, the dropwise addition of 7.77 g pure ethyl formate through the septum was administered. The reaction mixture was stirred over night at ambient temperature [1, 2].

On the next day, the reaction mixture has become a cloudy-orange suspension, which was evaporated to dryness. The remaining yellow solid was colloidally dissolved in water. By the addition of 1 M HCl, the pH-value (beginning 9-10) could be changed to pH 4 and orange suspension becomes a milky white, before some white solids start to precipitate. 14.80 g could be obtained as an amber solid. The crude product was still wet. This material was purified by column chromatography on silica gel by using hexane-ethyl acetate (8:2) as an eluent. 12.0 g light beige solid could be obtained as a purified product (**85%**).

MS (ESI^+):
m/z = 402.94 (calculated MW: 402.57 g/mol)

R_f: 0.6, hexane/ethyl acetate 7:3, UV active (254 nm), and visualized by Seebach derivatization reagent.



STEP III: 2α-Methyl-androstan-17β-ol-3-one (3)

In this "one-pot-reaction"-step the conversion to the 1α-methyl-group is performed simultaneously with the cleavage of the protective group at the 17β-alcohol:

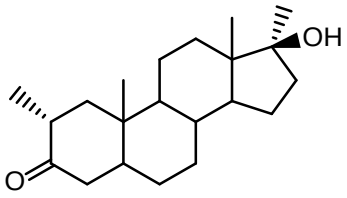
10.60 g Hydroxymethylene-precusor **2** was nearly dissolved in 800 ml ethanol at 55°C. This solution was hydrogenated in a pressure vessel (Parr reactor) with 6.00 g palladium on charcoal (5%) at 4 bar and room temperature for 24 h. The mixture was filtered twice through paper and the corresponding ethanol solution was evaporated, to yield 9.50 g colorless oil, still contains traces of charcoal and ethanol. The crude product was purified by column chromatography on silica gel by using hexane-ethyl acetate (8:2) as an eluent, to afford 5.45 g purified material as a colorless solid (**69%**) [1, 2].

MS (ESI^+):
m/z = 304.96 (calculated MW: 304.47 g/mol)

R_f: 0.35, hexane/ethyl acetate 7:3, visualized by Seebach derivatization reagent.

^1H NMR(300 MHz, DMSO-d_6) : δ 4.42 (d, 1 H, OH), 3.42 (m, 1H, H_{CHOH}), 3.32 (s, 1H, CH), 2.37 (t, 1 H, CH), 1.99 (dd, 1 H,), 1.84 (dd, 1H), 1.78 – 1.84 (m, 1H), 1.72 (m, 1H), 1.61 (m, 1H), 1.22-1.58 (m, 8H), 1.08 – 1.22 (m, 1H), 1.03 (s, 3H, CH_3), 0.76-1.01 (m + d, 7H, 2α-CH_3 +X), 0.65 – 0.72 (m, 1H), 0.64 (s, 3H, CH_3).

Preparation of methasterone:



2α-17α-Dimethyl-androstan-17β-ol-3-one (4)

2.05 g Oxymetholone was dissolved in 160 ml ethanol at room temperature. This solution was hydrogenated in a pressure vessel (Parr reactor) with 2.50 g palladium on charcoal (5%) at 3 bar and room temperature for 36 h. The reaction seemed to be completed, since there is no

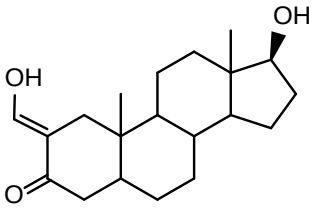
starting material remaining. The formation of two major byproducts can be observed via TLC (Seebach-staining). The mixture was filtered through a short pad of silica and the corresponding ethanol solution was evaporated to yield 1.90 g of a colorless solid [1, 2]. The crude product was purified by column chromatography on silica gel by using hexane-ethyl acetate (1:1), to afford 1.35 g of **4** as a colorless solid (**68%**).

MS (ESI^+):
m/z = 319.17 (calculated MW: 318.49 g/mol)

R_f: 0.80, hexane/ethyl acetate 1:1, visualized by Seebach derivatization reagent.

^1H NMR(300 MHz, $CDCl_3$-d_3) : δ 2.49 (sept, 1 H, CH), 2.34 (t, 1H, CH), 2.13 – 2.05 (m, 2H, CH_2), 1.87 – 1.25 (m, 12 H), 1.23 (s, 3 H, CH_3), 1.22 – 1.12 (m, 2H), 1.10 (s, 3 H, CH_3), 1.02 (d, 3 H, CH_3), 1.00 – 0.91 (m, 1 H), 0.89 (s, 3 H, CH_3), 0.88 – 0.80 (m, 1H), 0.71 (td, 1H, CH).

Preparation of 4,5α-dihydro-2-(hydroxymethylene)testosterone (oxystanolone):



2-Hydroxymethylene-androstan-17β-ol-3-one (5)

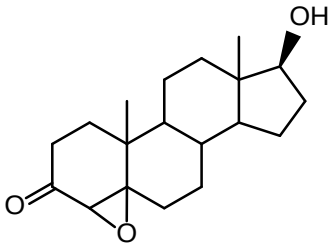
To a solution of 0.95 g intermediate (2) in 55 ml of the solvent mixture tetrahydrofuran/methanol/water 7:2:1 pyridinium tosylate (1.15 g dissolved in 20 ml tetrahydrofuran/methanol/water 7:2:1) was added at room temperature. Subsequently, this was followed by 8 h stirring at 50°C. Monitoring via TLC still shows remaining starting material [3].

After evaporation of all solvents, the crude product was chromatographed on silica gel by using hexane-ethyl acetate (8:2). 0.385 g of **5** was obtained as a colorless solid (**52%**).

MS (ESI^+):
m/z = 318.95 (calculated MW: 318.45 g/mol)

R_f: 0.35, hexane/ethyl acetate 7:3, UV active (254 nm), and visualized by Seebach derivatization reagent.

Preparation of clostebol:



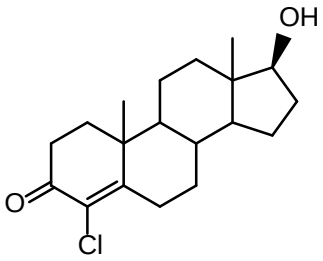
STEP I: 4,5-Epoxy-androstan-17β-ol-3-one (6)

10.0 g Testosterone was dissolved in 200 ml methanol and cooled to 0°C on ice. After cooling down, a chilled sodium hydroxide solution (1.40 g in 20 ml) was added dropwise, followed by the addition of 8 ml cold 30% hydrogen peroxide solution. The reaction was then stirred for 12 h at 0°C.

The reaction solution was then diluted with 500 ml water, before the resulting suspension was extracted several times with 200 ml dichloromethane. The combined organic phases were dried over Magnesium sulfate and all solvents were removed under vacuum [4]. This resulted in 8.50 g of **6** as a colorless foam (**85%**).

For further conversion to Clostebol (7), this intermediate was taken without any additional purification.

R_f: 0.45, hexane/ethyl acetate 1:1, visualized by Seebach derivatization reagent.



STEP II: 4-Chloro-androst-4-en-17β-ol-3-one (7)

8.50 g epoxide (6) was dissolved in 150 ml Acetone and treated with 14 ml of 25% hydrochloric acid. An immediate color shift of the reaction mixture from light yellow to deep blue can be observed. After stirring for 30 h at room temperature, a complete conversion of the epoxide (6) can be monitored by TLC. The reaction mixture was diluted with 400 ml water, leading to the formation of a blue-black solid precipitating from the solution.

After evaporation of acetone, the remaining aqueous solution was extracted several times with 200 ml dichloromethane. The combined organic extracts were washed with saturated sodium bicarbonate solution and sodium chloride solution before drying over magnesium sulfate.

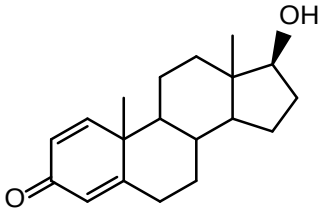
The crude product was chromatographed on silica gel by using hexane-ethyl acetat (1:1) and followed by a recrystallization from acetone [4]. The isolated product **7** was obtained as yellowish crystals (4.50 g; **51%**).

MS (ESI^+):
m/z = 322.83 + 324.80(^{37}Cl) (calculated MW: 322.87 g/mol)

R_f: 0.45, hexane/ethyl acetate 4:6, UV active (254), and visualized by Seebach derivatization reagent.

^1H NMR(300 MHz, DMSO-d_6) : δ 4.48 (d, 1 H, OH), 3.44 (m, 1H, H_{CHOH}), 3.05 (m, 1H, 2-CH_2), 2.63 (m, 1H, 2-CH_2), 2.42 (m, 1H), 2.27 (m, 1H), 1.98 (m, 1H), 1.48 – 1.90 (m, 7H), 1.30 – 1.41 (m, 2H), 1.25 (m, 1H), 1.21 (s, 3H, CH_3), 0.84 – 1.03 (m, 4H), 0.69 (s, 3H, CH_3).

Preparation of boldenone:



1,4-Androstdien-17β-ol-3-one (8)

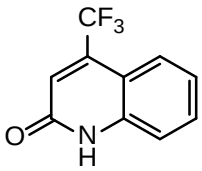
29.0 g Testosterone was dissolved in 220 ml dioxan/THF 8:2 and cooled to 0°C on ice. After cooling down a solution of tert-butyldimethylsilyl chloride (46.0 g in 100 ml dioxan/THF 8:2) was added dropwise and stirred for 90 min at 0°C. Then a suspension of 32.0 g of DDQ in 200 ml dioxan/THF 8:2 was added in 4 equal portions over a period of 4 h. The reaction mixture was stirred for additional 12 h within coming from 0°C to room temperature.
The resulting suspension was then filtered over Celite and rinsed with 300 ml THF. The filtrate was evaporated and brown oil was obtained. The oil was diluted in 1500 ml DCM and washed with 500 ml of 5% aqueous sodium hydroxide. The yellow organic phase was washed with 400 ml water and 400 ml saturated sodium chloride solution, before drying over magnesium sulfate.

After evaporation, the crude product was chromatographed on silica gel by using hexane-ethyl acetate (3:7), followed by a recrystallization in ethyl acetate [5]. 16.50 g of **8** was obtained as an amber solid (**58%**).

MS (ESI^+):
m/z = 287.20 (calculated MW: 286.41 g/mol)

R_f: 0.30, hexane/ethyl acetate 3:7, UV active (254 nm), and visualized by Seebach derivatization reagent
^1H NMR(300 MHz, $CDCl_3$) : δ 7.05 (d, 1 H, H_{olefin}), 6.23 (d, 1 H, H_{olefin}), 6.07 (s, 1 H, H_{olefin}), 3.64 (t, 1H, H_{CHOH}), 2.42 – 2.51 (m, 1H), 2.32 – 2.39 (m, 1H), 2.02 – 2.12 (m, 1H), 1.91-1.99 (m, 1H), 1.84 – 1.90 (m, 1H), 1.56 – 1.80 (m, 4H), 1.41 – 1.51 (m, 2H), 1.28 – 1.39 (m, 1H), 1.24 (s, 3H, CH_3), 0.91 – 1.14 (m, 4H), 0.82 (s, 3H, CH_3).

Preparation of LGD-2226:

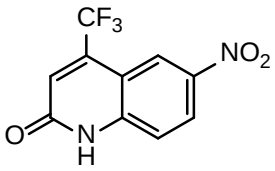


STEP I: 4-Trifluoromethylquinolin-2(1H)-one (9)

In a 1 L round flask fitted with a reflux condenser a mixture of 25.60 g aniline, 50.0 g ethyl-4,4,4-trifluoroacetoacetate 300 ml toluene was heated to reflux in an oil bath at 130 °C. After 20 min 3 ml water was added and the mixture was heated at reflux for another 24 h. the reaction mixture was cooled to room temperature and concentrated under reduced pressure. A round flask with 200 mL H_2SO_4 was heated to 80°C and the crude oil from the step before was added in portions to the H_2SO_4 keeping the internal temperature below 90°C, total addition time was approximately 40 min. After addition was complete, the mixture was stirred at 80°C for 1 h, cooled and poured onto 400 g crushed ice. The resulting solids were filtered, washed with water, and dried under vacuum at 40°C to give 33.0 g (**50%**) product (9) as a colorless solid [6, 7].

MS (ESI^+):
m/z = 214.13 (calculated MW: 213.16 g/mol)

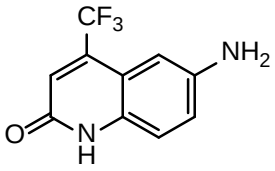
R_f: 0.10, hexane/ethyl acetate 7:3, UV active (254 nm).



STEP II: 6-Nitro-4-trifluoromethylquinolin-2(1H)-one (10)

A milky suspension of 33.0 g in 200 mL H_2SO_4 was cooled in an ice-salt bath to -5°C. 14.3 g 70% HNO_3 was added dropwise, keeping the internal temperature below 7°C. After addition was complete, the mixture was stirred at 0-10°C for 1 h and then after removal of the ice bath the temperature raised to room temperature within the next hours. The yellow solution was then poured onto 600 g crushed ice and kept standing/stirring for the next 12 h. The resulting two phases were separated and the organic phase equals a yellow slurry, which was filtered by suction. The resulting solid was washed with water and dried under vacuum. After crystallization of 20 g crude product in 1 L ethanol a nearly colorless solid (10) (**43%**) was obtained [6, 7].

MS (ESI^-):
m/z = 257.08 + 514.96 (2*M) (calculated MW: 258.15 g/mol)

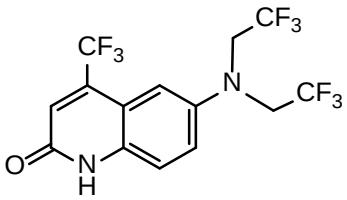


STEP III: 6-Amino-4-trifluoromethylquinolin-2(1H)-one (11)

A suspension of the 17.0 g Nitro-compound (10) in 400 ml ethanol in a 1 L round flask was flushed with argon and 2.0 g 5% Pd/C was added. The mixture was stirred under a hydrogen atmosphere for 2 days. Then filtered through silica and washed with 1500 mL ethanol. The filtrate was evaporated under vacuum to yield 14.0 g of a bright yellow solid 11 (**92%**).

Since the resulting solid has been monitored by TLC to exhibit high purity, the product was used without further purification for the next step.

MS (ESI^+):
m/z = 229.11 (calculated MW: 228.17 g/mol)



STEP IV: 6-N,N-Bis(2,2,2-trifluoroethyl)amino -4-trifluoromethylquinolin-2(1H)-one (12)

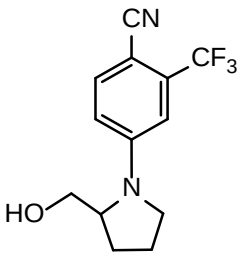
A 2 L round flask, under argon, was charged with a solution of the 14.00 g amine 11 in 350 ml trifluoroacetic acid. 5.80 g $NaBH_4$ was added within 1 h. The mixture was stirred at room temperature for 16 h. After that 4.50 g $NaBH_4$ was added and then heated to 70°C for 5 h. The heating bath was removed and it was stirred at room temperature over 2 d. In several portions water was carefully added. In total 1500 ml water was slowly added. The yellow-greenish precipitate was filtered and rinsed with water.

After drying, the crude product was purified by column chromatography on silica gel by using hexane-ethyl acetate (1:4), followed by a recrystallization in chloroform [6]. 18.1 g of **12** was obtained as a bright yellow solid (**76%**).
MS (ESI^+):
m/z = 392.86 (calculated MW: 392.22 g/mol)

R_f: 0.35, hexane/ethyl acetate 1:4, UV active (254 nm).

^1H NMR(300 MHz, DMSO-d_6) : δ 12.2 (bs, 1 H, NH), 7.57 (dd, 1H, H_{arom}), 7.39 (d, 1H, H_{arom}), 7.16 (s, 1 H, H_{arom}), 6.97 (s, 1 H, H_{olefin}), 4.39 (q, 4H, CH_2).

Preparation of LGD-4033:



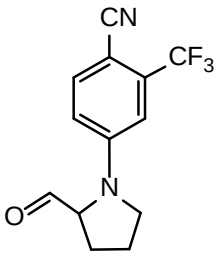
STEP I: 4-(2-(hydroxymethyl)-pyrrolidin-1-yl)-2-(trifluoromethyl)benzonitril (13)

10.0 g 4-Fluoro-2-(trifluoromethyl)benzonitril was dissolved in 200 ml tetrahydrofuran and 9.0 ml Hünig base was added dropwise. While stirring at room temperature, 5.0 g D-prolinol diluted in 100 ml tetrahydrofuran was added over a period of 15 min. The reaction mixture was stirred for 2 d at room temperature.

After evaporation, the crude product appears as a yellow oil, which was chromatographed on silica gel by using hexane-ethyl acetate (1:1) as a eluent to yield 11.40 g of **13** as a light yellow oil (**88%**) [8].

MS (ESI^+):
m/z = 270.89 (calculated MW: 270.25 g/mol)

R_f: 0.30, hexane/ethyl acetate 1:1, UV active (254 nm).



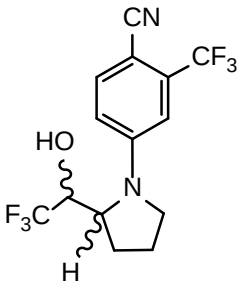
STEP II: 4-(2-(formylpyrrolidin-1-yl)-2-(trifluoromethyl)benzonitril (14)

6.99 g Oxalylchloride was diluted in 100 ml dichloromethane and cooled down to -78°C by dry ice/acetone bath. 9.83 g dimethylsulfoxide diluted in 50 ml dichloromethane was added via syringe over a period of 30 min. After stirring 20 min at -78°C, 11.4 g intermediate-alcohol 13 diluted in 60 ml dichloromethane is added dropwise over a period of 45 min. After stirring 45 min at -78°C, the addition of 29 ml triethylamine starts, following by 30 min stirring at -78°C.

Then the ice bath was removed and the reaction mixture was stirred 12 h at room temperature. The resulting yellow-milky suspension was stirred with 200 ml saturated sodium chloride solution. After the separation of the organic phase, the aqueous phase was extracted two times with 100 ml dichloromethane. The combined organic phases were dried over magnesium sulfate and evaporated. The resulting yellow solid was purified by column chromatography on silica gel by using hexane-ethyl acetate (1:1) as a eluent [8]. 11.20 g of **14** was obtained as a yellow solid (**99%**).

MS (ESI^+):
m/z = 269.08 (calculated MW: 268.23 g/mol)

R_f: 0.40, hexane/ethyl acetate 1:1, UV active (254 nm).



STEP III: 4-(2-(1-Hydroxyl-2,2,2-trifluoroethyl)pyrrolidin-1-yl)-2-(trifluoromethyl)benzonitril (15)

11.20 g Aldehyde 14 was dissolved in 300 ml dry tetrahydrofuran and 8.36 g CsF was added directly as a solid to the reaction mixture. It was cooled via ice bath to 0°C and stirred for 15 min. Then 25.00 g trimethyl(trifluoromethyl)silane was added via syringe over a period of 40 min. The reaction is stirred for 15 h coming from 0°C to room temperature over this time interval. The reaction solution shifts color from nearly colorless to dark brown.

For stopping the reaction, it was diluted with 100 ml saturated ammonium chloride solution and then evaporated. The resulting emulsion was extracted several times with ethyl acetate and the combined phases dried over magnesium sulfate. After evaporation of all volatiles, the intermediate silyl-ether can be obtained as red-brown oil.

This intermediate was converted to the final product **15** without any further purification.

The intermediate was diluted with 400 ml tetrahydrofuran and cooled to 0°C. A cooled potassium hydroxide solution (4.76 g in 400 ml water) was mixed with the diluted oil and stirred at 0°C for 2h. After quenching with 300 ml water, the organic solvent was removed via evaporation from the mixture. The remaining brown oil was extracted with dichloromethane and dried over magnesium sulfate, to yield a reddish-brown oil.

This crude product was chromatographed on silica gel by using hexane-ethyl acetat (8:2) as an eluent. During purification, different fractions with several product isomers can be collected [8].

The isolated products were obtained as 8.50 g yellowish oils (**61%**).

MS (ESI^+):
m/z = 338.97 (calculated MW: 338.25 g/mol)

R_f: 0.65 + 0.50 (isomers), hexane/ethyl acetate 1:1, UV active (254 nm + 366 nm).

^1H NMR(300 MHz, DMSO-d_6): δ 7.85 (d, 1H, H_{arom}), 6.94 (d, 1H, H_{arom}), 6.87 (dd, 1 H, H_{arom}), 6.53 (bd, 1 H, OH), 4.28 (m, 1H), 4.20 (m, 1H), 3.55 (m, 1H), 2.14 – 2.25 (m, 2H), 1.89 – 2.02 (m, 2H).

Conflicts of Interest

Matthias Grill was an employee of Lipomed AG, Switzerland. The other authors declare no conflict of interest.

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