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# Highly efficient, solvent-free esterification of testosterone promoted by a recyclable polymer-supported tosylic acid catalyst under microwave irradiation

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**Dedicated to Professor Michał Fedoryński – a fabulous academic tutor and propagator of organic chemistry among talented young people – on the occasion of his 73nd birthday and retirement**

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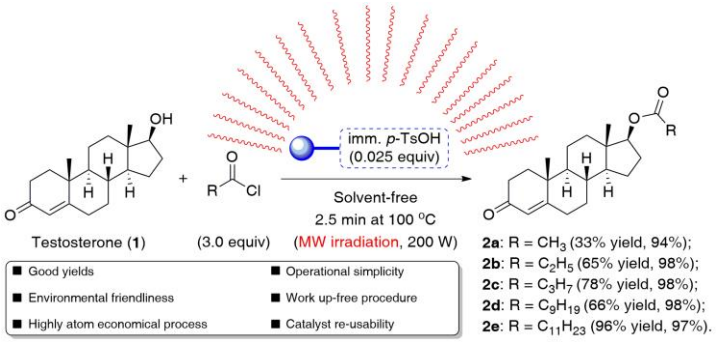
## Abstract

Although the classical acylation of testosterone clearly benefits from a broad substrate scope and available catalysts, the requirement of hazardous reagents and the high waste production are its drawbacks. To optimize the process efficiency as well as minimize the environmental impact, we decided to develop a novel method of testosterone esters synthesis, which relies on the usage of recyclable heterogeneous polymer-supported tosylic acid catalyst and microwave-assistance effect in a non-solvent system. Under the established MW-conditions, the acceleration of the process rate was so efficient that the reaction completed within 2.5 min, thus affording the desired esters in the 33–96% yield range without using a work-up procedure. Furthermore, the elaborated catalytic system could be recycled for at least 2 runs not only without a loss of the products yield, but unexpectedly with significant improvement of the reaction efficiency, which may indicate that the reduction of the catalyst loading is possible. We believe that this finding constitutes a very good starting-point for further optimization of the studied process.




**Keywords:** Heterogeneous catalysis, polymer-supported tosylic acid, testosterone esters, esterification, microwave-mediated reaction, green chemistry

## Introduction

Testosterone (**1**, androst-4-en-17β-ol-3-one or 17β-hydroxyandrost-4-en-3-one), the major male sex hormone naturally secreted by Leydig cells in the testes, is an organic molecule important not only from a physiological point of view [as it is essential for male sexual differentiation, growth and function of the male genital tract, masculinized secondary sexual characteristics, sexual potency, production of spermatozoa (spermatogenesis) and male fertility], but also from the perspective of medicinal chemistry and drug design.[1] Therefore, testosterone provides a key starting material for various active pharmaceutical ingredients (API) of many registered drugs, which are most often used to treat a variety of endocrine disorders, such as male hypogonadism (hypoandrogenism)[2–4] and hypoactive sexual desire disorder in women (HSDD).[5–8] While testosterone constitutes a prominent example of an anabolic-androgenic steroid (AAS) substance, it is also utilized for distinct androgen-requiring therapies along with male-hormone replacement therapy (M-HRT),[9–11] female androgen deficiency syndrome (FAIS) therapy,[12–15] and the masculinizing hormone therapy.[16,17] Moreover, various novel testosterone derivatives have been proven beneficial for successful treatments of other diseases including certain types of *carcinoma* (especially breast and prostate cancers),[18–20] anemia,[21,22] muscular dystrophies[23–25] and osteoporosis.[26–29] Since it is well-recognized that relatively high doses of exogenous testosterone suppress spermatogenesis, there have been reports on the synthesis and experimental use of various testosterone derivatives in male hormonal contraception.[30–34] In addition, because of high anabolic efficiency of testosterone, a plethora of its derivatives are unfortunately extensively employed in non-medical use, e.g. as athletic performance-enhancing drugs in sport doping trades.[35] For example, since the 1950s elite athletes of various professional sport disciplines have used supra-physiologic doses of testosterone preparations to improve their strength and stamina, whereas bodybuilders mainly to enhance their net skeletal muscle mass development.[36] However, the great majority of contemporary AAS users are not competitive athletes and they risk their health and life buying these drugs on the black market and using them primarily for personal appearance and rejuvenating effect.[37]

It is apt at this point to mention that testosterone itself is not applied as API molecule because the pure unmodified steroid has a poor bioavailability and is rapidly metabolized in the liver (ca. 2 hours) into an inactive form.[38] In order to prevent biological elimination of testosterone-based drugs *in vivo*, and thus increase their pharmacological activity, the molecular structure of the crude steroid is predominantly functionalized toward more stable compounds. In this regard, the respective ester-like derivatives (obtained by acylation of testosterone at 17β-hydroxy position with fatty acids of various aliphatic and/or aromatic chain lengths) are the most common prodrugs of testosterone. The pharmaceuticals manufactured on the basis of long-acting testosterone esters resistant toward metabolic destruction and possessing high bioavailability can affect the human organism with different pharmacokinetic profiles for up to 2–12 weeks after *intra musculum* administration depending on the formulation of a particular preparation as well as its dosing regimens.[39] Of course, the shorter the aliphatic chain of the ester moiety in such a testosterone derivative, the shorter the elimination half-life of the particular compound. Some of the most prominent examples of testosterone-like drugs marketed at present are depicted in Figure 1.

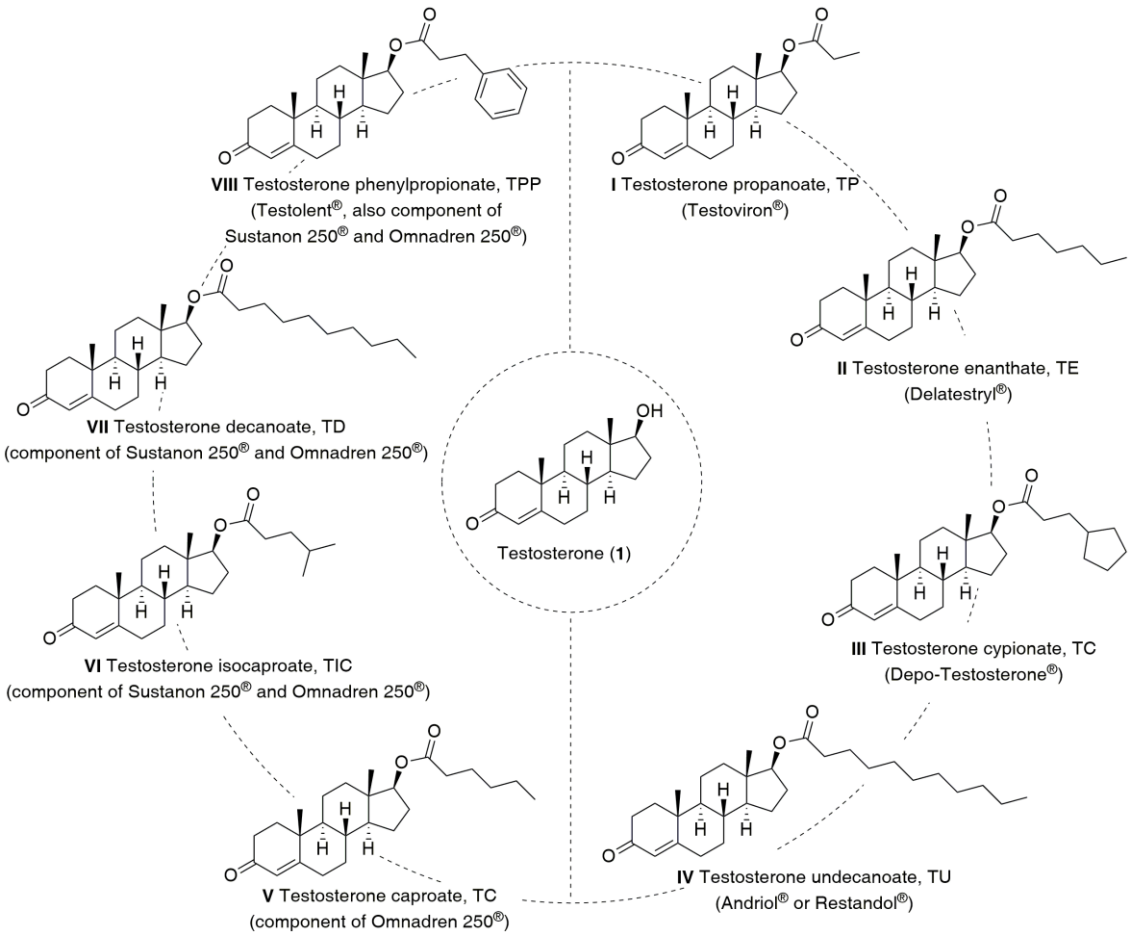



**Figure 1.** Representative chemical structures of esters of testosterone used as FDA-approved APIs (**I**–**IV**) and nonprescription AAS (**V**–**VIII**), illegally sold through the black market.

In view of the medicinal importance of testosterone and its economic impact on the pharmaceutical industry, synthetic studies on the preparation of its 17β-ester derivatives have attracted considerable interest. Consequently, a plethora of chemical protocols have been explored to provide routes toward process improvements of an esterified variants of testosterone. Amongst the most common methods are those which employ activated carboxylic acid derivatives such as acid anhydrides or acid chlorides in the presence of basic amines such as triethylamine ($Et_3N$),[40,41] pyridine (Py),[42–49] 4-(dimethylamino)pyridine (DMAP)[50] and/or *N*,*N*-dimethylaniline (DMA)[51] as well as a binary mixture of those amines (Py/DMAP),[52–57] respectively. A similar kind of conversions have been reported by Yadav et al.[58] using potassium fluoride on alumina (KF-$Al_2O_3$) as a solid base catalyst.

In addition to the above-mentioned dual active nucleophilic-basic catalyst systems, acidic montmorillonite[59] as well as (Lewis acid)-type copper(II) trifluoromethanesulfonate [$Cu(OTf)_2$][60] catalysts are also known to efficiently promote the esterification of testosterone with the respective acyl-group-donor regents (acid anhydrides/chlorides). Notably, it is somehow interesting that no data are provided concerning strong mineral acids ($H_2SO_4$, HCl etc.) as the catalysts routinely used in a conventional Fischer–Speier esterification approach. This might suggest that testosterone is acid-sensitive and that the esters are not affordable this way possibly because of rearrangement, self-etherification and/or dehydration of the starting material. The synthetic strategies which rely on carbodiimides activators are also worth mentioning. In this regard, Fuji and co-workers[61] reported that acylation of testosterone can proceed smoothly thanks to the

employment of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC×HCl) and DMAP as the base. In extension to the above work, several years later Morales-Serna et al.[62] elaborated a mild, high-yielding method that utilized carboxylic acids and a combination of EDC and 1-hydroxybenzotriazole (HOBt) as the coupling reagents in the presence of the catalytic amount of DMAP or calcined hydrotalcite [$Mg_6Al_2CO_3(OH)_{16}$×4($H_2O$)]. In turn, Kenda et al.[63] used *N*,*N*'-dicyclohexylcarbodiimide (DCC) along with DMAP to obtain 17β-monoacylated testosterone esters. Very recently for the same purpose the reaction systems composed of DCC/DMAP additives were employed by independent groups of researchers.[64–67] On the other hand, testosterone functionalization toward ester-like derivatives can also be accomplished by means of μ-oxo-tetranuclear zinc clusters [$Zn_4(OCOCF_3)_6O$][68,69] or Zr-containing metal–organic framework (MOF) nanocrystals, namely (Zr)UiO-66($NH_2$)/$SiO_2$ hybrid materials.[70]

Each of the methods listed-above has its own particular pros and cons, however, it is worth pointing out that although syntheses of testosterone esters catalyzed by amines proceed in good yields, the application of these compounds is rather limited because of extremely flammable and corrosive triethylamine, highly toxic and expensive DMAP or pyridine, harmful to men's fertility. Furthermore, although carbodiimide-like reagents furnish an impressive coupling efficiency and mild reaction conditions, the major drawback of using them is their high price, difficult-to-remove impurities (e.g. dicyclohexylurea) and strong skin allergy inducing potency. In the case of KF-$Al_2O_3$-mediated reactions, testosterone is acylated almost quantitatively with AcCl in 2.5 h, however, KF must be used in significant molar excess and if it leaches off the surface of alumina, it can etch silicate glasses due to the formation of soluble silicon fluorides. In turn, using tetranuclear zinc clusters of Zr-based MOF nanocrystals is rather expensive, as their availability is low and the rates of the reactions are sluggish (i.e. the MOF-catalyzed transformation was conducted at 75 °C and accomplished with the desired testosterone caprylate after as long as 48 h) and require huge 20-fold molar excess of caprylic acid. Unfortunately, a common disadvantage for most of the reported methods is the need to use volatile and flammable organic solvents, which makes them prone to generating hazardous and irksome waste streams and thus unfeasible for the large-scale production.

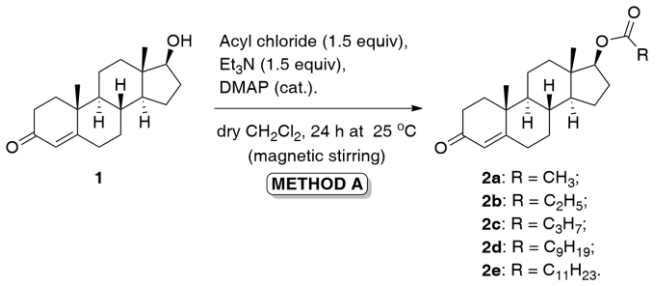
In view of all the above aspects, it is more than certain that new attention to the development of improved and environmentally benign synthetic methods of obtaining testosterone ester-like derivatives is required. Therefore, in this work our ultimate goal was to develop a novel and facile synthetic procedure for testosterone esters preparation based on simple, straightforward, low-cost and environmentally-benign procedure using recyclable polymer-supported tosylic acid catalyst under solvent-free microwave irradiation conditions.

## Results and Discussion

Herein, we report our results on devising a novel esterification method for the preparation of 17β-acylated testosterone derivatives. At the outset of our investigations, the reference compounds required for analytical purposes were synthesized using standard synthetic methodology. In this regard, in a model acylation reaction, testosterone (**1**) was treated with 1.5 equiv. of the respective acyl chloride (i.e. acetyl, propionyl, butyryl, decanoyl and dodecanoyl chloride) in the presence of 1.5 equiv. of $Et_3N$ as a base and catalytic amount of DMAP diluted in anhydrous $CH_2Cl_2$ at 25 °C (Method A). The reactions were completed in 24 h time span, and the acylated products **2a**–**e** were isolated in the range of 41–87% yield and purity >95% in accordance with HPLC analysis (Table 1).

**Table 1.** Synthesis of the testosterone esters **2a**-**e** using acid chlorides and $Et_3N$/DMAP catalytic system (Method A)[a]




| Entry | Product | Yield [%][b] | Purity [%][c] | $[\alpha]_D$[d] |
|---|---|---|---|---|
| 1 | **2a** | 41 | 93 | +101.00 |
| 2 | **2b** | 72 | >99 | +98.00 |
| 3 | **2c** | 61 | 97 | +103.00 |
| 4 | **2d** | 87 | 99 | +95.00 |
| 5 | **2e** | 76 | >99 | +95.00 |

[a] Reaction conditions: **1** 200 mg, acyl chloride (1.5 equiv.), $Et_3N$ (1.5 equiv.), DMAP (cat.), dry $CH_2Cl_2$, 15 min at 0–5 °C, then 24 h at 25 °C, 800 rpm (magnetic stirrer).

[b] Isolated yield after purification by silica gel-packed column chromatography.

[c] According to HPLC analysis.

[d] Specific rotation, *c* solution in chloroform (*c* 0.50), *T* = 29.0 °C.

The assigned structures of the obtained esters **2a**–**e** were established from their spectral properties ($^1H$ and $^{13}C$ NMR, HRMS, IR and UV-VIS), melting point characteristic, specific rotation values and finally by comparison with the available literature data. It is also worth to note that the reactions based on a classical $Et_3N$/DMAP catalytic system mostly proceeded very cleanly without the formation of isomerization by-products. However, in the case of testosterone acetate (**2a**) it turned out that the desired ester was contaminated with side products, which were inseparable *via* the convenient $SiO_2$-column chromatography probably due to close physical properties proximity furnishing the same $R_f$-value.

In searching for a novel, efficient method of testosterone esters synthesis we have encountered the paper of Temperini et al.,[71] who functionalized various alcohols acylating them with isopropenyl acetate and catalytic *para*-toluenesulphonic acid (*p*-TsOH) under mild conditions. At first glance, it seemed a very interesting alternative, especially because of the fact that the enol esters guarantee irreversibility of the reaction since the resultant enolates rapidly tautomerise *in situ* into a more stable keto compounds [acetaldehyde (in the case of vinyl esters) or acetone (in the case of isopropenyl esters) or ethyl acetate (in the case of ethoxyvinyl esters)], which are unable to react in the opposite direction shifting the reaction's thermodynamic equilibrium towards product formation. In addition to this crucial feature, when using low molecular enol esters the particular acylating agent itself as well as the generated side product, both being volatile, can be easily removed by evaporation under vacuum, thus simplifying or even entirely eliminating the work-up procedure. Moreover, enol esters are significantly less corrosive than the corresponding acyl chlorides. Following the discovery of these results, we tried to extend the methodology toward testosterone (**1**). However, in our hands, transesterification of the titled steroid **1** by 4 equiv. of the appropriate enol ester and 0.02 equiv. of *p*-TsOH in $CH_2Cl_2$ or $CH_3CN$ suggested in the cited paper failed as the starting material was

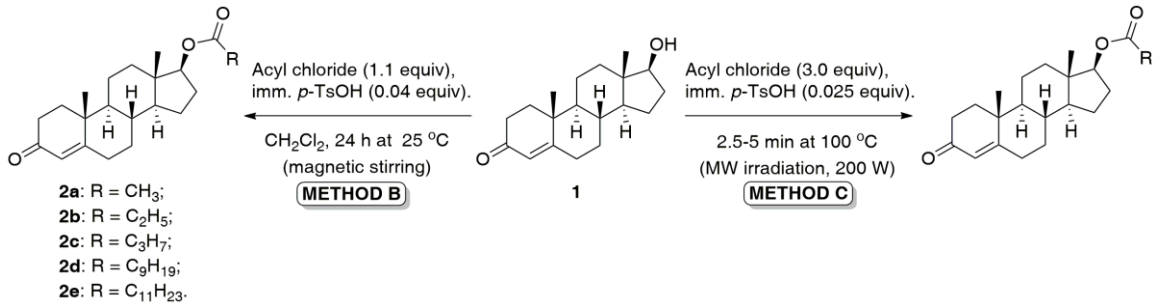
recovered unchanged almost quantitatively. We have also assessed the possibility of carrying out these reactions without the solvent and with significantly prolonged reaction time, however, there was no conversion of **1** into **2a**–**e** probably because of the limited solubility of the substrate in neat enol esters. In turn, when the same reaction was investigated under microwave-assisted conditions a plethora of by-products was observed, and among them no ester formation could be detected. Other reported variation of this approach, which assumes the employment of enol esters in the presence of a catalytic amount of iodine[72] was deliberately rejected by us since it was proved by Ahmed et al.[73] to be incompatible with another androstane derivative, namely 19-nortestosterone (nandrolone), leading to simultaneous acetylation of the 3-ketone group due to enolizable carbonyl function.

In the forward direction to synthesize testosterone esters, we focused our efforts on catalytic behaviour of tosylic acid. Moreover, inspired by the well-established fact that microwave irradiation provides excellent rate acceleration and thus often dramatically reduces reaction times,[74–76] we decided to implement this strategy to our studies as well. Interestingly, the utilization of microwave-assisted heating in the preparation of testosterone esters still remains unexplored, and only scant data in the literature can be found to date. Moreover, the conditions of the already reported attempts seems to be far from optimal since i.e. Penov Gaši et al.[77] reported microwave-assisted synthesis of testosterone salicylate in poor 35% yield obtainable from the reaction carried out with highly hazardous metallic sodium for 30 min at 200 °C. A slightly shorter reaction time (20 min) was required for MW-heating set at 230 °C to synthesised 17'-succinimidyltestosterone in excellent 96% yield.[78] In turn, Marwah and co-workers[79] achieved efficient acetylation of various sterols (including **1**) in semisolid state using acetic anhydride ($Ac_2O$) and a catalytic amount of toluene-*p*-sulfonic acid monohydrate (*p*-TsOH×$H_2O$) under microwave irradiation. Although the esterification of **1** proceeded rapidly (2 min), the MW dielectric heating effect is hard to define in this case, because the reactions were carried out using a domestic MW oven. The utilization of household microwave unit in the organic synthesis suffers from unfocused/dispersed irradiation causing uncontrolled heating and low reproducibility, a potentially serious fire hazard, and an inability to stir reactions during irradiation resulting in splashing of the reagents when conducted in an open-vessel type reactors such as a beaker or test tube. Moreover, the extension of the established conditions toward other steroidal esters could be somehow problematic, since acid anhydrides are significantly less available than acid chlorides. And last but not least, the authors elaborated their methodology on the basis of *p*-TsOH×$H_2O$, thus precluding the catalyst recovery and recycling.

With this in mind, in the present study, we selected polymer-bound macroporous *p*-TsOH instead of the native monohydrate catalyst. It was also dictated by the desire to limit the moisture in the catalytic system, which was expected to be beneficial for the reaction's outcome in terms of preventing the irreversibility of the process or side reactions (i.e. dehydration or acid-mediated racemisation). In this regard, two independent synthetic strategies relying on immobilized *p*-TsOH and acyl chlorides have been examined in terms of the efficiency of the reaction conditions. Therefore, we compared the reaction durations as well as the yields and purity of the synthesized esters for both conventional and MW-assisted methods (Table 2).

**Table 2.** Synthesis of the testosterone esters **2a**–**e** using acid chlorides and immobilized *p*-TsOH in conventional (conductive) heating (Method B) and microwave (MW) irradiation (Method C) reaction modes




| Entry | Product | Conventional heating (Method B)[a] | | MW-assisted heating (Method C) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 5 min[b] | | 2.5 min[c] | |
| | | Yield [%][d] | Purity [%][e] | Yield [%][d] | Purity [%][e] | Yield [%][d] | Purity [%][e] |
| 1 | **2a** | 74 | 97 | 23 | 91 | 33 | 94 |
| 2 | **2b** | 62 | 90 | 28 | 97 | 65 | 98 |
| 3 | **2c** | 70 | 98 | 50 | 98 | 78 | 98 |
| 4 | **2d** | 42 | 95 | 44 | 95 | 66 | 98 |
| 5 | **2e** | 60 | 91 | 93 | 97 | 96 | 97 |

[a] Reaction conditions: **1** 150 mg, acyl chloride (1.1 equiv.), immobilized *p*-TsOH (0.04 equiv.), $CH_2Cl_2$, 24 h at 25 °C, 800 rpm (magnetic stirrer).
[b] Reaction conditions: **1** 150 mg, acyl chloride (3.0 equiv.), immobilized *p*-TsOH (0.025 equiv.), 5 min at 100 °C, 200 (W).
[c] Reaction conditions: **1** 150 mg, acyl chloride (3.0 equiv.), immobilized *p*-TsOH (0.025 equiv.), 2.5 min at 100 °C, 200 (W).
[d] Yields refer to isolated products purified by silica gel-packed column chromatography.
[e] According to HPLC analysis.

Yet another difference between those two attempts also taken into account, was the reaction medium. The conventionally-heated reactions were carried out in a regular organic solvent, and MW-mediated reactions were carried out in neat conditions. Of course, before starting those experiments, we had also attempted blind reactions without using the catalyst in order to established non-catalytic effect of both the examined systems. The results of control-experiment trials were negative, as the reactions furnished only traces of products. At this stage of synthetic studies additional simple 'leaching experiments' have been carried out to find out if the esterifications catalyzed by immobilized *p*-TsOH are heterogeneous in nature. Thus, the suspension of the polymer-supported tosylic acid catalyst in dry $CH_2Cl_2$ was stirred for 24 h at 25 °C without addition of the reagents. After filtration of the *p*-TsOH-beads, an aliquot was allowed to stand for an additional 2 h, and then both reagents [testosterone (**1**) and acetyl chloride (1.1 equiv.)] were introduced to the pretreated $CH_2Cl_2$-solution followed by 24-h reaction. The results of this trial were negative as only negligible amounts of the product **2a** were observed according to HPLC. Since no additional reaction takes place under homogeneous conditions in solution these observations support the essential role played by *p*-TsOH as a truly heterogeneous catalyst. Only then, we have initially performed a reaction between testosterone (**1**) and 1.1 equiv. of the respective acyl chloride using catalytic amount of immobilized *p*-TsOH

suspended in $CH_2Cl_2$ at 25 °C under conventional thermal heating conditions (Method B). The progress of the reactions was monitored by TLC. As shown in Table 2, all the proceeded reactions were virtually completed after 24 h in moderate [**2d** (42%), **2e** (60%), **2b** (62%)] to good [**2c** (70%), **2a** (74%)] isolated yields.

The conventional chemical reaction workup based on tedious, time- and labor-consuming liquid−liquid extraction (LLE) often involves the use of relatively large volumes of solvents and possible emulsion formation disrupting the separation between liquid phases. Therefore, it must be noted herein that we decided to verify if using immobilized tosylic acid catalyst could facilitate the replacement of the LLE procedure by a simple filtration. Although the reaction mixture contained excess amounts of the employed reagent (acyl chloride), and certainly acidic wastes as well, we did not destroy them by quenching the filtrate with a suitable $NaHCO_3$ aqueous solution, but further checked if a wet-loading of the crude oil residue directly onto a chromatographic column packed with $SiO_2$ would affect the purification procedure. Fortunately, it turned out that the elimination of the reaction workup did not blemish chromatographic separation, and most of the products, including acetate **2a**, butanoate **2c** and decanoate **2d**, could be successfully afforded in very high purity (>95%) according to HPLC analysis (see Supporting Information). Disappointingly, propionate **2b** and laurate **2e** were significantly more contaminated, and thus the purity of the isolated esters did not exceeded 92% after the application of the established chromatographic separation conditions.

In the next step of our optimization studies we examined the effect of microwave irradiation on esterification of testosterone (**1**). For this purpose, some changes within the reaction conditions had to be introduced. Because it is well documented that steroids possessing enolizable carbonyls are prone to undergoing 3-enol-ester formation in the presence of higher amounts of acidic catalysts, to eliminate this effect responsible for side reaction, we therefore decreased molar excess of *p*-TsOH from 0.04 equiv. to 0.025 equiv. as suggested by Marwah et al.[79] In addition, a minimum of 3-fold molar excess of liquid acid chlorides was required to cover up and fully moisten solid-state substrate **1** in order to make a paste sufficient to obtain effective mass transfer of the reactants and the catalyst leading to the completion of the desired acylation. Interestingly, if the composed reaction mixtures were irradiated in a microwave oven for 5 min with additional stirring and power set at 200 W with maximum temperature of 100 °C, it turned out that the synthesized esters could be obtained with high rate of conversion. However, except for dodecanoate **2e**, which was isolated in excellent 93% yield, the rest of the esters were furnished in poor [**2a** (23%), **2b** (28%)] to fair [**2d** (44%), **2c** (50%)] yields.

As the presence of tautomerizable α,β-unsaturated 3-ketone functionality in testosterone molecule (**1**) often causes low reaction control in terms of chemoselectivity, resulting in reduced overall yields of the desired mono-substituted 17β-esters, it was worth studying how the reaction time can influence the efficiency of our process when exposed to acidic conditions. Therefore in the next step, a series of testosterone's straight-chain aliphatic esters **2a**–**e** were synthesized under the specified reaction conditions and reduced to 2.5 min MW irradiation time. As before, the products were isolated from the crude reaction mixtures without using biphasic liquid-liquid extractions. The results summarized in Table 2 demonstrate that the majority of the esters **2b**–**e** were isolated in high yields (65-96%) and excellent purity (>97%). The afforded yield enhancement might be due to more selective acylation of testosterone (**1**) at the 17β-hydroxyl group than in the approaches involving longer reaction times. The worst results in terms of the reaction yield (33%) and chemical purity (94%) were again observed for testosterone acetate (**2a**). In this case, the caused rapid MW-assisted reaction gave a difficult to separate complex mixture of 17β-acetylated and 3-enol-acetylated products. Nevertheless, it is worth noting that in the case of MW-assisted reactions an admirable 576-fold rate enhancement is attainable when compared to conventional heating attempts. The characterization data of the

obtained esters **2a**–**e** matched that of what was previously reported in literature and those obtained *via* classical route using ($Et_3N$/DMAP)-catalytic system.

The beneficial effects of microwave-assisted heating versus conventional heating were also observed when the recyclability of the catalyst was studied using as a model reaction consisting of testosterone (**1**) and 3-fold molar excess of propionyl chloride (Figure 2). It is somehow interesting that regardless of the reaction time, the yields increased within the subsequent charges, being as follows: 28% (I), 44% (II), and 53% (III) for 5 min approaches, and 65% (I), 68% (II) and 73% (III) for 2.5 min approaches, respectively.

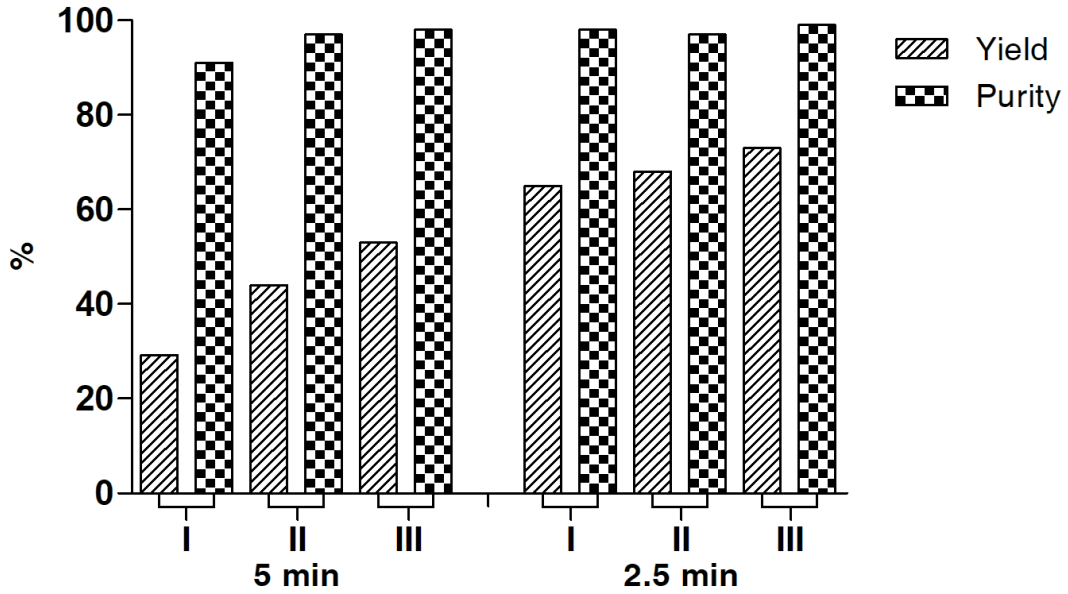



**Figure 2.** Studies on recycling of the immobilized *p*-TsOH catalyst in the microwave-assisted synthesis of testosterone propionate (**2b**) carried out for 5 min and 2.5 min, respectively. The catalyst reuse was performed with propionyl chloride (3 equiv.) as the acyl donor. Purity of the ester **2b** was assessed by HPLC analysis.

Noteworthy, especially in the case of 5 min reactions, a rapid activity improvement after the first reaction was noted. Moreover, the loss of the catalyst's mass during the subsequent reaction cycle was noticed to be ca. 1 mg (~ 15%), but this did not affect the catalytic activity and consequently good yields were attained. As it was already stated above, the removed *p*-TsOH catalyst after intensive washing with acetone was then dried on standing before using it in the next reaction cycle assessed after following the night break. Based on these results, it should be mentioned that since the storage stability of *p*-TsOH preparation is very high and the catalyst itself is not a hygroscopic material, it might be also suitable for use in humid climates.

Because of both attempts (reactions lasting 2.5 min and 5 min) finished within the complete consumption of the substrate **1** according to TLC indications, there might be a two-fold plausible explanation of these results. On the one hand, this seemingly unusual behaviour can be attributed to the physical phenomena concerning structural modification of the polymer carrier invoked by MW-irradiation effect (this might facilitate diffusion rate of the reagents and the forming product to-and-from the catalyst particles/site located in-and-on porous material). On the other hand, a rational cause can be potentially related within a fall in the activity of tosylic acid preparation itself, which, when exploited during the subsequent reaction cycles, stops catalyzing parallel side reactions and/or secondary products degradation. If that was true, then the results presented above would reveal that utilizing even lower loading of the catalyst than the initial 0.025

equiv. might be beneficial for the process outcome. However, this phenomenon definitely needs more experimental efforts to be undertaken, and therefore has not been elaborated on in the present paper since it would definitely exceed the frame of this work.

## Conclusions

We have developed an efficient, swift, cheap and environmentally friendly method for esterification of the testosterone molecule based on polymer-supported tosylic acid heterogeneous catalyst under solvent-free MW-irradiation assisted conditions. The elaborated process was improved in terms of the operational simplicity (no need to use dry conditions or a gas-protecting atmosphere), cost-efficiency [proven recyclability of the catalyst and extremely short reaction time afforded (2.5 min)] as well as elimination of hazardous organic solvents typically used in conventional esterification methodologies. The great benefit of this strategy is that the laborious work-up is not required, which stems from the fact that the solid catalyst is truly heterogeneous and can be removed by simple filtration, while the permeate after being concentrated under vacuum is directly subjected on $SiO_2$-packed column chromatography to isolate pure steroid esters. Although within the established methodology testosterone acetate was afforded in low yield (33%), fortunately during the synthesis of long chain aliphatic esters the selectivity of acylations was significantly improved, and thus the formation of complicated mixtures of side-products was abandoned allowing us to obtain the desired products in >65% isolated yield. Therefore it might be concluded that this method is sufficient only for longer chain aliphatic testosterone esters synthesis as the acetyl chloride is too reactive in the presence of acidic catalyst leading to less selective transformations. The developed work-up-free microwave-enhanced esterification procedure may provide an alternative highly atom economical process for the chemical industry. Further investigations on the recyclability of polymer-supported tosylic acid and the relation between acidity of this catalyst and its potency in catalyzing testosterone esterification as well as the extension of this methodology toward wider scope of more challenging substrates are currently on-going and will be reported in due course.

## Experimental Section

**General.** Reagents and solvents were purchased from various commercial sources (Sigma Aldrich, Alfa Aesar, POCH) and were used without further purification. Testosterone [purum, ≥99.0% (HPLC)] was purchased from Sigma Aldrich (Cat. No.: 86500 Sigma); immobilized *p*-TsOH (immobilized *p*-toluenesulfonic acid polymer-bound macroporous, 30-60 mesh, extent of labeling: 2.0-3.0 mmol/g loading) was purchased from Sigma Aldrich (Cat. No.: 532312 Sigma). Methylene chloride was dried by allowing it to stand over activated (oven-roasted in high-vacuum) 3Å molecular sieves [20% mass/volume (m/v) loading of the desiccant] at least for 48 h before use.[80] All reactions, which needed anhydrous conditions (non-aqueous reactions), were carried out under an atmosphere of dry argon using flame-dried glasswares. Evaporation of the solvent residues was performed at reduced pressure by means of Büchi rotary evaporator. Melting points, uncorrected, were determined with a commercial apparatus (Thomas-Hoover "UNI-MELT" capillary melting point apparatus) on samples contained in rotating glass capillary tubes open on one side (1.35 mm inner diam. and 80 mm length). Analytical thin-layer chromatography was carried on TLC aluminum plates (Merck) covered with silica gel of 0.2 mm thickness film containing a fluorescence indicator green 254 nm ($F_{254}$), and using UV light as a visualizing agent. Preparative separations were carried out by column chromatography using thick-walled glass

columns and silica gel (230–400 mesh) with grain size 40–63 μm or activated charcoal as stationary phase, respectively. Optical rotations ([$\alpha$]) were measured with a PolAAr 32 polarimeter in a 2 dm long cuvette using the sodium D line (λ=589 nm); the units of the specific rotation are: (deg×mL)/(g×dm). The chromatographic analyses (HPLC) were performed with a Shimadzu CTO-10ASV chromatograph equipped with STD-20A UV detector and Chiralcel OD-H chiral column (4.6 mm×250 mm, 5 μm grain size from Daicel Chemical Ind., Ltd.) and with a pre-column (4 mm×10 mm, 5 μm) using mixtures of *n*-hexane/2-PrOH as mobile phase in the appropriate ratios given in experimental section [both the mobile phase composition as well as the flow rate were fine tuned for each analysis (see Table S1 in the Supporting Information)]; the wavelength of UV detection was set at 254 nm; the HPLC analyses were executed in an isocratic and isothermal (30 °C) manner. UV spectra were measured with Cary 3 spectrometer; samples were dissolved in absolute EtOH. $^{1}$H NMR (500 MHz) and $^{13}$C NMR (126 MHz) spectra were recorded on a Varian NMR System 500 MHz spectrometer; $^{1}$H and $^{13}$C chemical shifts ($\delta$) are reported in parts per million (ppm) relative to the solvent signals [$CDCl_3$, $\delta_H$ (residual $CHCl_3$) 7.26 ppm, $\delta_C$ 77.00 ppm]. Chemical shifts are quoted as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br s (broad singlet); coupling constants (*J*) are reported in Hertz. Mass spectrometry was recorded on Micro-mass ESI Q-TOF spectrometer [ESI, ESI-HRMS: additives of mixtures of $CH_3CN$/MeOH/$H_2O$ (50:25:25, v/v/v) + 0.5% formic acid and MSI concept 1H (EI, 70eV ionization)]. Microwave heating was performed in a single-mode microwave reactor (CEM Discover LabMate) equipped with a calibrated infrared (IR) temperature sensor using closed-vessel setting.

**Classical method for the preparation of testosterone esters 2a–e. Method A.** To a solution of testosterone (**1**, 200 mg, 0.69 mmol) in dry $CH_2Cl_2$ (10 mL), $Et_3N$ (104 mg, 1.04 mmol, 1.5 equiv) and DMAP (15 mg, 0.12 mmol) were added. The mixture was cooled to 0–5 °C in ice bath. Next, one of the appropriate acyl chloride (1.5 equiv) was dissolved in dry $CH_2Cl_2$ (5 mL), and added dropwise to the reaction mixture by using syringe. Afterward, the cooling bath was removed, and the resulting mixture was stirred for 24 h at 25 ºC. The crude mixture was diluted with $CH_2Cl_2$ (20 mL), subsequently quenched with $H_2O$ (40 mL), the water phase was extracted with $CH_2Cl_2$ (3 × 20 mL), and the combined organic layer was washed with saturated water solution of $NaHCO_3$ (80 mL), brine (80 mL), and dried over anhydrous $MgSO_4$. After evaporation of the residuals of solvent under reduced pressure the crude product was purified by column chromatography on silica gel, using gradient of mixture of $CHCl_3$/acetone (98:2, 95:5, v/v), thus obtaining desired esters **2a**–**e** with 41–87% yield, respectively.

**Tosylic acid-catalyzed synthesis of testosterone esters 2a–e under conventional heating (Method B) and microwave irradiation (Method C). Method B.** To a solution of testosterone (**1**, 50 mg, 0.17 mmol) in $CH_2Cl_2$ (0.7 mL) the appropriate acyl chloride (1.1 equiv) and immobilized *p*-TsOH (3.5 mg, 6.8 μmol, 0.04 equiv) were added. The reaction mixture was stirred for 24 h at 25 °C. Next, the crude reaction mixture was dissolved in acetone (3 mL), the immobilized catalyst was removed by filtration under suction, and the resulting permeate was evaporated to dryness using rotapovator. The crude oil residues were purified by silica gel column chromatography using gradient of mixture of $CHCl_3$/acetone (99:1, 98:2, 95:5, v/v) to afford the testosterone esters **2a**–**e** in 42–74% yield, respectively. Physical and spectral data of **2a**–**e** were consistent with the one reported in literature. The blank reactions (without catalyst) were also run in this case.

**Method C.** Testosterone (**1**, 150 mg, 0.52 mmol), immobilized *p*-TsOH (6.5 mg, 13 μmol, 0.025 equiv), and the appropriate acyl chloride (3.0 equiv) were placed in a glass microwave vial (10 mL) capped with a Teflon septum, and subjected to microwave irradiation with an initial ramp time of 30 sec. at 35 °C (200 W). The

temperature was then increased to 100 °C, using a 200 W MW-source with a holding time of 2.5 or 5 min, respectively (depending on the experiment performed, maximum pressure inside the MW reactor was in the range of 2-4 bars). Afterwards, the reaction was stopped by bringing the content of the vial to room temperature by cooling the jet for 1 min. Next, the crude reaction mixture was dissolved in acetone (3 mL), the immobilized catalyst was removed by filtration under suction, then rinsed with additional portion of acetone (3 mL), and the resulting permeate was evaporated to dryness using rotapovator. The crude oil residues were purified by silica gel column chromatography using gradient of mixture of $CHCl_3$/acetone (99:1, 98:2, 95:5, v/v) to afford the 17β-testosterone esters **2a**–**e** in 23–96% yield, respectively. Physical and spectral data of **2a**–**e** were consistent with the one reported in literature and those obtained *via* classical route using ($Et_3N$/DMAP)-catalytic system. Attention: The removed *p*-TsOH catalyst after washing with acetone was dried on standing and used directly in the next charge without additional treatment.

**Testosterone acetate (2a).** mp 135.5–136 °C ($CHCl_3$/acetone) [Lit.:[81] 136–137 °C (no data)]; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.81 (s, 3H), 0.82–1.11 (s, 3H), 1.16 (s, 3H), 1.26–1.92 (m, 10H), 2.01 (s, 3H), 2.09–2.48 (m, 6H), 4.57 (dd, *J*=9.3, 7.8 Hz, 1H), 5.69 (s, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 12.0, 17.4, 20.5, 21.1, 23.4, 27.5, 31.5, 32.7, 33.9, 35.4, 35.7, 36.6, 38.6, 42.4, 50.2, 53.7, 82.4, 123.9, 170.9, 171.1, 199.4 [The NMR spectral data were accordance with those reported in the literature[42]]; IR (nujol): $\nu_{max}$ = 1736, 1664, 1618, 1445, 1329, 1267, 1248, 1182, 1116, 1044, 1025, 935, 862, 725; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{21}H_{31}O_3^+$ *m/z*: 331.2273, Found 331.2151; $[\alpha]_D^{29}$ = +101.00 (*c* 0.50, $CHCl_3$) {Lit.[82] $[\alpha]_D$ = +93.10 (*c* 1.00, $CHCl_3$) or lit.:[62] $[\alpha]_D^{25}$ = +82.30 (*c* 1.00, $CHCl_3$)}; HPLC [*n*-hexane-2-PrOH (85:15, v/v); f = 0.8 mL/min; λ = 254 nm]: $t_R$= 12.792 or [*n*-hexane-2-PrOH (90:10, v/v); f = 0.8 mL/min; λ = 254 nm]: $t_R$ = 16.736 or [*n*-hexane-2-PrOH (95:5, v/v); f = 0.8 mL/min; λ = 254 nm]: $t_R$ = 26.785; UV/VIS: $\lambda_{max}$ = 242 nm (EtOH).

**Testosterone propionate (2b).** mp 121–122 °C ($CHCl_3$/acetone) [Lit.:[83] 121–123 °C (no data)]; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.83 (s, 3H), 0.90–1.24 (m, 9H), 1.28–1.90 (m, 10H), 1.97–2.05 (m, 1H), 2.11–2.46 (m, 7H), 4.60 (dd, *J*=9.2, 8.0 Hz, 1H), 5.72 (d, *J*=0.7 Hz, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 9.24, 12.0, 17.4, 20.5, 23.5, 27.5, 27.8, 31.5, 32.7, 33.9, 35.4, 35.7, 36.6, 38.6, 42.5, 50.3, 53.7, 82.2, 123.9, 170.9, 174.5, 199.4 [The NMR spectral data were accordance with those reported in the literature[84]]; IR (nujol): $\nu_{max}$ = 1740, 1725, 1681, 1668, 1452, 1270, 1229, 1188, 1076, 1041, 1019, 870, 714; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{22}H_{33}O_3^+$ *m/z*: 345.2425, Found 345.2173; $[\alpha]_D^{26}$ = +98.00 (*c* 0.50, $CHCl_3$) {Lit.:[46] $[\alpha]_D^{25}$ = +63.00 (*c* 2.30, $CHCl_3$)}; HPLC [*n*-hexane-2-PrOH (85:15, v/v); f = 0.8 mL/min; λ = 254 nm]: $t_R$ = 11.618; UV/VIS: $\lambda_{max}$ = 244 nm (EtOH).

**Testosterone butanoate (2c).** mp 107–110 °C ($CHCl_3$/acetone) [Lit.:[85] 108.85 °C (no data)]; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.81 (s, 3H), 0.92 (t, *J*=3.4 Hz, 3H), 0.95–1.10 (m, 1H), 1.16 (s, 3H), 1.26–1.90 (m, 13H), 1.95–2.05 (m, 1H), 2.09–2.45 (m, 8H), 4.59 (t, *J*=8.6 Hz, 1H), 5.70 (s, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 12.0, 13.6, 17.4, 18.5, 20.5, 23.5, 27.5, 31.5, 32.7, 33.9, 35.4, 35.7, 36.4, 36.6, 38.6, 42.5, 50.2, 53.7, 82.1, 123.9, 170.8, 173.6, 199.3 [The NMR spectral data were accordance with those reported in the literature[46]]; IR (nujol): $\nu_{max}$ = 1724, 1668, 1615, 1414, 1351, 1267, 1229, 1188, 1132, 1101, 1047, 1013, 941, 864; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{23}H_{35}O_3^+$ *m/z*: 359.2586, Found 359.2327; $[\alpha]_D^{29}$ = +103.00 (*c* 0.50, $CHCl_3$) {Lit.:[46] $[\alpha]_D^{25}$ = +78.00 (*c* 1.71, $CHCl_3$)}; HPLC [*n*-hexane-2-PrOH (85:15, v/v); f = 0.8 mL/min; λ = 254 nm]: $t_R$ = 10.752; UV/VIS: $\lambda_{max}$ = 246 nm (EtOH).

**Testosterone decanoate (2d).** mp 53–54.5 °C ($CHCl_3$/acetone) [Lit.:[83] 55–57 °C (no data)]; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.83 (s, 3H), 0.87 (t, *J*=6.9 Hz, 3H), 0.91–1.11 (m, 3H), 1.18 (s, 3H), 1.20–2.53 (m, 32H), 4.60 (t, *J*=8.8 Hz, 1H), 5.72 (s, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 12.0, 14.1, 17.4, 20.5, 22.6, 23.5, 25.1, 27.5, 29.1, 29.2, 29.2, 29.4, 31.5, 31.8, 32.7, 33.9, 34.6, 35.4, 35.7, 36.6, 38.6, 42.5, 50.3, 53.7, 82.1, 123.9, 170.9, 173.8, 199.4 [The NMR spectral data were accordance with those reported in the literature[46]]; IR (nujol): $\nu_{max}$ = 1721, 1680,

1618, 1417, 1333, 1273, 1226, 1172, 1116, 1069, 1041, 1010, 941, 869, 1736; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{29}H_{47}O_3^+$ *m/z*: 443.3525, Found 443.3252; $[\alpha]_D^{29}$ = +95.00 (*c* 0.50, $CHCl_3$) {Lit.:[46] $[\alpha]_D^{25}$ = +74.00 (*c* 0.99, $CHCl_3$)}; HPLC [*n*-hexane-2-PrOH (85:15, v/v); f = 0.8 mL/min; λ = 254 nm]: $t_R$ = 8.715; UV/VIS: $\lambda_{max}$ = 239 nm (EtOH).

**Testosterone laurate (2e).** mp 64–65 °C ($CHCl_3$/acetone) [Lit.:[46] 146–149 °C ($EtOH_{aq.}$)]; $^1$H NMR (500 MHz, $CDCl_3$): *δ* 0.83 (s, 3H), 0.85–0.90 (m, 3H), 0.91–1.09 (m, 4H), 1.18 (s, 3H), 1.22–1.44 (m, 17H), 1.46–1.89 (m, 10H), 1.98–2.06 (m, 1H), 2.17 (m, 1H), 2.24-2.47 (m, 6H), 4.60 (dd, *J*=9.1, 7.8 Hz, 1H), 5.72 (s, 1H); $^{13}$C NMR (126 MHz, $CDCl_3$): *δ* 12.1, 14.1, 17.4, 20.5, 22.7, 23.5, 25.1, 27.5, 29.1, 29.2, 29.3, 29.4, 29.6 (2C), 31.5, 31.9, 32.7, 33.9, 34.6, 35.4, 35.7, 36.6, 38.6, 42.5, 50.2, 53.7, 82.1, 123.9, 171.0, 173.9, 199.4 [The NMR spectral data were accordance with those reported in the literature[46]]; IR (nujol): $\nu_{max}$ = 1736, 1680, 1624, 1261, 1232, 1172, 1118, 1064, 1045, 1013, 946, 870, 726; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{31}H_{51}O_3^+$ *m/z*: 471.3833, Found 471.3446; $[\alpha]_D^{29}$ = +95.00 (*c* 0.50, $CHCl_3$) {Lit.:[46] $[\alpha]_D^{25}$ = +73.00 (*c* 1.19, $CHCl_3$)}; HPLC [*n*-hexane-2-PrOH (85:15, v/v); f = 0.8 mL/min; λ = 254 nm]: $t_R$ = 8.372; UV/VIS: $\lambda_{max}$ = 240 nm (EtOH).

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## Supplementary Material

Supplementary data associated with this article can be found in the online version and contains copies of $^1$H and $^{13}$C NMR, HRMS, IR and UV/VIS spectra as well as HPLC chromatograms.

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