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Facile Ecofriendly Synthesis of Monastrol and Its Structural Isomers via Biginelli Reaction

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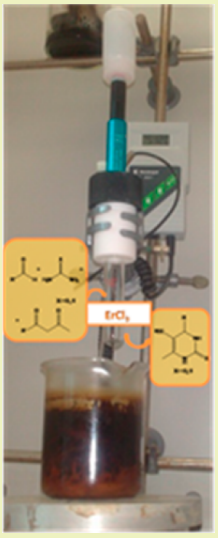
Supporting Information

ABSTRACT: In this paper, a Q-tube equipment is proposed as a valid alternative to monomode MW technology to synthesize in a simple and economic way a library of dihydropyrimidine derivatives performing the Biginelli reaction in solvent-free conditions under pressure and with the catalysis of a very mild and environmentally benign Lewis acid consisting of erbium trichloride hexahydrate.



KEYWORDS: *Monastrol, Biginelli reaction, Q-tube reactor, Microwaves, Lanthanides*

INTRODUCTION

Over a century ago, the Italian chemist Pietro Biginelli discovered a multicomponent reaction that produced 4-aryl-3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) in a simple one-pot condensation between ethyl acetoacetate, benzaldehyde, and urea under strongly acidic conditions.[1] In the last two decades, DHPMs and their derivatives have been receiving considerable attention due to the wide range of biological effects including antiviral, antitumor, antibacterial, and anti-inflammatory activities described for these compounds.[2−6] Moreover, the importance of this pharmacophore has been further increased by the identification of the (±)-4-(3-hydroxyphenyl)-2-thione derivative named Monastrol,[7] as a potential lead compound for the development of new anticancer drugs (Figure 1).

Unfortunately, the original protocol of the Biginelli reaction, which employs a strong acidic catalyst,[1] gives only a low yield of the dihydropyrimidine derivatives, particularly in the case of substituted aromatic and aliphatic aldehydes.[8] Hence, in order to improve the efficacy of this reaction, several improved procedures have been reported in the past decade.[9−12]

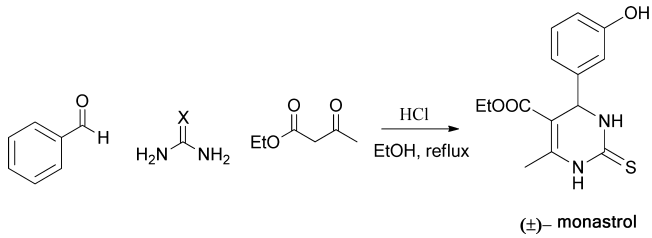


Figure 1. Biginelli reaction for the synthesis of (±)-monastrol.

Our research group has been long involved in developing green chemical methodologies for the organic synthesis, so our attention was attracted by the reported methods for performing the Biginelli reaction avoiding the use of harmful solvents and/or employing nonconventional technologies.[9−17] From this point of view, the use of microwave activation to perform the Biginelli reaction in a few minutes,[15] and the adoption of environmental friendly systems such as a nontoxic recyclable

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catalyst under solvent-free conditions,[12,13] were particularly attractive. Along this line, it is worth noting that several groups have developed solvent-free syntheses of Monastrol and other dihydropyrimidine derivatives.[9,18,21−24] Liu and Wang[12] reported the first solvent- and catalyst-free Biginelli reaction applied to the synthesis of dihydropyrimidines. Significantly, they highlighted the difficulties in using hydroxylbenzaldehyde when Lewis acids were used as catalysts, so they proposed that a catalyst-free method could avoid this shortcoming even though the yields obtained were lower than those observed in the presence of a catalyst and solvent.

In continuation of our green chemistry program,[25−31] we present a simple and economic method to synthesize a library of dihydropyrimidinone derivatives performing the Biginelli reaction under pressure under solvent-free conditions and with the catalysis of a very mild and environmentally benign Lewis acid.

EXPERIMENTAL METHODS

All chemicals were obtained from Aldrich Chem. Co. or Acros Organics and used as received. All solvents were distilled using standard methods before use. MW-assisted reactions were performed on a Synthos 3000 instrument from Anton Paar, equipped with a 64MG5 Rotor and an IR probe used for external temperature control. Q-tube assisted reactions were performed in a Q-tube safe pressure reactor from Q Labtech, equipped with a cap/sleeve, pressure adapter (120 psi), needle adapter/needle, borosilicate glass tube, Teflon septum, and catch bottle. Reactions were monitored using a GC−MS Thermo Fisher Scientific workstation, composed of a Focus GC (Thermo TR 5 ms SQC 15 m × 0.25 mm ID × 0.25 μm, working on split mode, 1.2 mL/min using He as the carrier gas) and a DSQ II mass detector. TLC was performed using Kielsegel 60-F264 on aluminum plates, commercially available from Merk. Liquid flash chromatography was performed on a Supelco VERSA FLASH HTFP station using silica cartridges commercially available from Supelco. HR-MS measurements were realized on a Thermo Scientific Q Exactive (Thermo Fisher, Milan, Italy) mass spectrometer working in positive mode at 70,000 resolving power, operating in SIM mode by flow injection (flow rate 15 μL/min for each stock solution). Stock solutions at a concentration of 1 mg/L were prepared in UHPLC-MS grade MeOH for each analyte separately. Prior to analysis, each solution was diluted 1:1000 (v/v) in a vial to obtain a concentracion of 1 mg/mL. Detection of the targeted compounds was based on theoretical exact mass. Data were evaluated by Xcalibur 2.2.SP1 (Thermo Fisher Scientific, Bremen, Germany). The mass accuracy, directly calculated from Xcalibur, is defined by the formula Δ(ppm) = [(theoretical mass − measured mass)/theoretical mass] × 1.000.000. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WM 300 instrument on samples dissolved in DMSO or other specified solvents when necessary. Chemical shifts are reported in parts per million (ppm) from tetramethylsilane as the internal standard for ^{1}H and ^{13}C NMR spectra (0.0 ppm). Coupling constants (J) are reported in Hertz. All unknown products in this report were characterized by standard techniques (^{1}H NMR and ^{13}C NMR, GC/MS), while for the known products, the data were compared with those reported in the literature for identification.

General Procedures for Q-Tube-Assisted Synthesis of DHPMs. Q-tube assisted reactions were performed in a Q-tube safe pressure reactor from Q Labtech, equipped with a cap/sleeve, pressure adapter (120 psi), needle adapter/needle, borosilicate glass tube, Teflon septum, and catch bottle. Urea or thiourea (1.0 mmol), aldehyde (1.0 mmol), and a dicarbonyl compound (1.0 mmol) were sequentially added in a 12 mL Q-tube pressure tube, furnished by Q Labtech. $ErCl_3{\cdot}6H_2O$ (5.0 mol %) was added in the experiments conducted in the presence of a catalyst. A Teflon septum was placed on the top of the tube, and an appropriate cap and pressure adapter were used. The mixture was heated in an oil bath at 120 °C. After about 30 min, the reaction mixture was monitored by TLC and GC/MS and stopped. The obtained solid reaction mixture was worked up as described below.

General Procedures for MW-Assisted Synthesis of DHPMs. MW-assisted reactions were performed on a Synthos 3000 instrument from Anton Paar, equipped with a 64MG5 Rotor and an IR probe used for external temperature control. Urea or thiourea (1.0 mmol), aldehyde (1.0 mmol), and dicarbonyl compound (1.0 mmol) were sequentially added in 3 mL glass vial using a Synthos 3000 microwave oven (Anton-Paar). $ErCl_3{\cdot}6H_2O$ (5.0 mol %) was added in the experiments conducted in the presence of a catalyst. Then, the appropriate Teflon and screw caps were placed on the top of the vial, and the mixture was heated in the MW reactor at 120 °C in "power-controlled mode" (P_{max} 600W). After 30 min, the reaction was controlled by GC/MS revealing that the conversion was not complete. After about 90 min, the reaction mixture was monitored by TLC and GC/MS and stopped. Then, the obtained solid reaction mixture was worked up as described below.

1Ta, **1Ua**, **1Tb**, **1Ub**, **3Ta**, **3Ua**, **5Ta**, and **5Ua**: The solid reaction mixture was poured onto crushed ice and stirred for 5−10 min. The solid was separated by filtration and washed with water/ethanol (2:1) and dried under vacuum. Characterization of known products (**1Ta**, **1Ua**, **1Tb**, **1Ub**, **3Ta**, **3Ua**) was made by comparison with the data reported in the literature.[6,9,24,32−35] Novel products (**5Ta**, **5Ua**) were characterized by ^{1}H NMR, ^{13}C NMR, and GC/MS (Supporting Information). All products were obtained as a racemic mixture.

2Ta, **2Ua**, **4Ua**, and **4Ta**: The solid reaction mixture was poured onto crushed ice and stirred for 5−10 min. The gummy residue was separated by decantation and suspended in ethyl acetate to separate urea or thiourea as solid. Then, the filtered solution was evaporated under vacuum, and the crude compound was purified by flash chromatography (Supporting Information). Characterization of known products (**2Ua**) was made by comparison with the data reported in the literature.[11] Novel products (**2Ta**, **4Ua**, **4Ta**) were characterized by ^{1}H NMR, ^{13}C NMR, and GC/MS (Supporting Information). All products were obtained as a racemic mixture.

2Ub and **2Tb**: The solid reaction mixture was poured onto crushed ice and stirred for 5−10 min. The aqueous solution was extracted three times with chloroform, and the combined organic phases were dried with Na_2SO_4 and evaporated in a vacuum. The crude compound was purified by flash chromatography (Supporting Information). Pure products were characterized by ^{1}H NMR, ^{13}C NMR, and GC/MS (Supporting Information). All products were obtained as a racemic mixture.

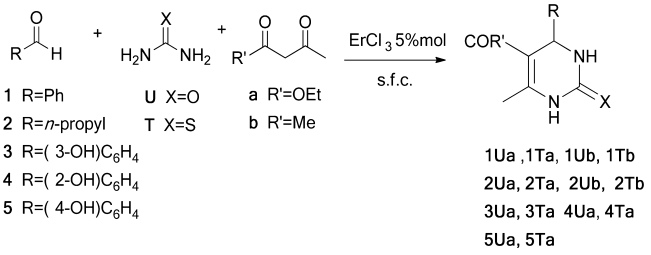
RESULTS AND DISCUSSION

As reported, ethyl 4-(3-hydroxyphenyl)-6-methyl-2-sulfanylidene-3,4-dihydro-1*H*-pyrimidine-5-carboxylate (Monastrol, Figure 1) is a specific inhibitor of the BimC class kinesin Eg5,[7] which has permitted more critical analyses of Eg5 function during spindle assembly[36,37] and as a reversible agent to synchronize cells in metaphase.[38] The numerous attempts to modify the Monastrol structure, although it fails to improve its inhibition activity against Eg5, emphasizes the presence of H-bond donor/acceptor groups on the aromatic ring and is crucial for the interaction with the active site of the enzyme.[6,9,32−34] Hence, in designing our experimental work, we focused our attention on Monastrol and its positional structural isomers. The significant results reported for the microwave-assisted Biginelli reactions is the reduction of reaction time[19−24] together with the chance to perform the reaction in solvent-free conditions (s.f.c.), suggesting the potential of the Q-tube system to perform the Biginelli reaction under similar conditions. The Q-tube is a safe pressure reactor that features a pressure release and reseal system (patent pending) that prevents accidental explosions due to overpressurization. Q-tube is an affordable alternative to expensive and cumbersome MW synthesizers; as a MW reactor, this system enables a

reaction to be carried out at a higher temperature than the boiling point of the solvent and in the presence of a reagent, which will increase the reaction rate, even for the solvents that are MW transparent. One of the aims of the present report is to compare the efficacy of these two techniques in performing the Biginelli reaction.

First, we investigated the original Biginelli reaction of ethyl acetoacetate, benzaldehyde, and urea under solvent-free conditions and without the use of any catalyst (Scheme 1).

Scheme 1. Synthesis of DHPMs by Biginelli Reaction



This showed that only a modest yield of product **1Ua** was obtained both in the Q-tube and under microwave irradiation (Table 1, entry 1). Given the high efficiency of lanthanide(III) Lewis acids to catalyze solution-phase Biginelli condensation[35,39−43] and our past experience in the use of these salts,[25−31] we attempted to improve the performance of the process repeating the reaction under the same conditions but in the presence of a small amount of Er(III) chloride hexahydrate as catalyst. The results reported as entry 1 in Table 1 show the efficiency of erbium(III) chloride to promote the reaction under microwave activation as well in the Q-tube system giving a higher yield of product **1Ua** (Table 1, entry 1). These results clearly confirm the efficiency of Q-tube as a valid alternative technique, which provides a cleaner reaction profile very similar to what is observed in the MW-assisted protocol but in shorter reaction time. Moreover, it confirms the efficiency of the catalyst; the use of cheap and less toxicity $ErCl_3$ hexahydrate[44] as catalyst in solvent-free conditions permitted the sustainability of the whole process.

Encouraged by these results, we studied the Biginelli condensation of several aromatic and aliphatic aldehydes with 1,3-dicarbonyl compounds and urea and thiourea. We compared the yields obtained performing the reaction under microwave activation and in the Q-tube system with or without the addition of catalytic amounts of erbium(III) salt (Scheme 1). The results summarized in Table 1 show that the two methods worked well for both the urea (U) and thiourea (T) (e.g., Table 1, entries 1 and 2) as well as for ketoesters (a) and diketones (b) (e.g., Table 1, entries 1 and 3). The crucial role of erbium(III) chloride in improving the reaction performance was evident for all the reported examples, and in some cases, acceptable yields of product were obtained only in presence of the catalyst (Table 1, entries 6−8, 11, and 12). Notably, acceptable yields of dihydropyrimidine derivatives were registered also in the case of poorly reactive aliphatic aldehyde **2** (Table 1, entries 5−8) for which the use of the Q-tube system without catalyst was crucial for obtaining a significant improvement of the process in terms of yield. In the presence of the catalyst, yields obtained using the Q-tube technology were comparable with those obtained under MW with an improvement in terms of cleanness and byproducts reduction (see GC profiles in Supporting Information).

Table 1. Q-Tube vs MW-Assisted Biginelli Reactions

		Q-tube (30 min, 120 °C)		MW reactor (30 min, 120 °C)		MW reactor (90 min, 120 °C)	
entry	compound	conv. (%)[a]	yield (%)[a]	conv. (%)[a]	yield (%)[a]	conv. (%)[a]	yield (%)[a]
1	**1Ua**	60	45	50	30	76	62
		100[b]	92[b]	90[b]	50[b,c]	95[b]	80[b]
2	**1Ta**	60	40	70	35	75	60
		100[b]	90[b]	90[b]	45[b,c]	90[b]	78[b]
3	**1Ub**	60	45	60	0[c]	75	50
		100[b]	96[b]	90[b]	0[b,c]	90[b]	70[b]
4	**1Tb**	45	27	30	0	50	32
		100[b]	100[b]	70[b]	0[b,c]	70[b]	51[b]
5	**2Ua**	50	15[c]	0	0[d]	75	31[d]
		75[b]	64[b,c]	47[b]	5[b,c,d]	75[b]	54[b,d]
6	**2Ta**	30	0[c]	0	0[d]	50	0[d]
		70[b]	56[b,c]	35[b]	0[b,c,d]	55[b]	17[b,d]
7	**2Ub**	50	0[c]	50	0[c,d]	50	0[d]
		80[b]	56[b,c]	60[b]	0[b,c,d]	60[b]	26[b,d]
8	**2Tb**	50	0[c]	0	0[d]	50	0[d]
		80[b]	40[b,c]	60[b]	0[b,d]	60[b]	10[b,d]
9	**3Ua**	43	30	40	30	61	50
		80[b]	75[b]	70[b]	40[b,c]	78[b]	70[b]
10	**3Ta**	45	35	45	32 45[b,c]	65	52
		89[b]	80[b]	68[b]		82[b]	75[b]
11	**4Ua**	0	0	0	0	0	0
		54[b]	44[b]	0[b]	0[b]	55[b]	51[b]
12	**4Ta**	0	0	0	0	0	0
		65[b]	56[b]	0[b]	0[b]	62[b]	53[b]
13	**5Ua**	50	50	30	0[c]	80	70
		100[b]	90[b]	80[b]	0[b,c]	95[b]	87[b]
14	**5Ta**	70	42	25	0[c]	78	74
		100[b]	100[b]	100[b]	10[b,c]	100[b]	100[b]

[a]GC/MS data. [b]Reaction conducted in presence of 5 mol % of $ErCl_3$. [c]Aldol condensation intermediate was detected. [d]Self-condensation and/or polymerization byproducts were detected (Supporting Information).

In exploring the applicability of the compared methods, we particularly focused our attention on the synthesis of Monastrol **3Ta** and its aromatic positional isomers **4Ta** and **5Ta** as well as their oxo-analogues **3Ua**, **4Ua**, and **5Ua** (Scheme 1) that have not been tested as EG5 inhibitors yet. All these derivatives preserve an OH group on the aromatic ring that secures the presence of an H-donor/acceptor on the molecule with different electronic and polar features different from the original molecule Monastrol.

All Biginelli reactions reported in Table 1 were carried out employing equimolar amounts of reagents with or without the addition of 5.0 mol % of catalyst. The microwave activation of the reaction showed to be very useful, especially in the presence of the catalyst. Moreover, the comparison of the processes performed in the MW reactor and Q-tube reactor revealed that the latter gave analogous results but in faster reaction times and with cleaner reaction profiles, particularly evident in the case of unstable substrates such as butyraldehyde **2** (Table 1, entries 5−8). Remarkably, just in the case of the **2Ua** product, against a comparable conversion of the substrate, a higher yield of Biginelli products were obtained in the Q-tube reactor compared to MW-assisted processes, together with lower

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amounts of byproducts, as evidenced by GC/MS analysis (see chromatographic profile in Supporting Information).

In all the examples, the solid final reaction mixture was suspended in ice water and then filtered; finally, the obtained solids were recrystallized from water, ethanol, or a combination of both, while a chromatography purification was necessary for only the products **2Ta**, **2Ub**, **2Tb**, **4Ua**, and **4Ta**.

Specific attention was paid for Monastrol derivatives **4Ua** and **4Ta** that, contrary to common DHMPs, showed very high solubility in ethanol and methanol as well as very low tendency to precipitate from aqueous solutions. They were purified by flash column chromatography as inseparable mixtures of two tautomers (see Supporting Information for solvent systems), which were well separated and identified by GC/MS where, notably, different fragmentation profiles for each single component of the relative mixture were evident (see Supporting Information for details on isomeric ratio calculation, GC profiles, and EI/MS comments). Thus, in the case of compound **4Ua** (*m*/*z* 276), the fragmentation of the ring itself generates different ions, although the fragmentation profile relative to the substituents on the dihydropyrimidine ring were absolutely comparable ([M-Et]$^+$, [M-COOEt]$^+$, [M-PhOH]$^+$). Therefore, we hypothesized that the Biginelli product **4Ua** exists as a 64/36 mixture of two stable tautomeric isomers (isomer **I** and **II**, as depicted in the GC/MS chromatogram, Figure 2), whose different electronic structure could give the two different fragmentation pathways depicted in Figure 2.[44]

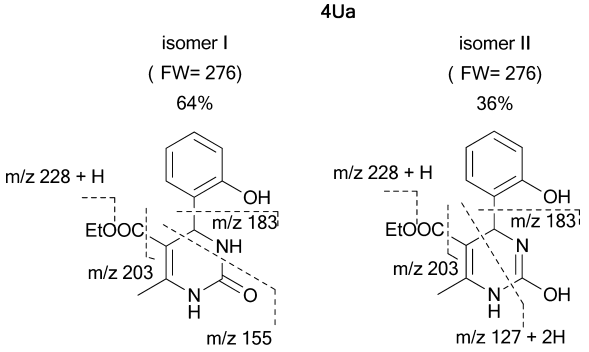

Figure 2. MS fragmentation of isomers **I** and **II** of compound **4Ua**.

Furthermore, similar differences in the MS spectra of product **4Ta**, the thio-analogue of **4Ua**, were observed. In the case of **4Ta**; however, a 9/91 mixture of tautomeric forms (isomer **I** and **II**) was hypothesized as shown in Figure 3. In both cases,

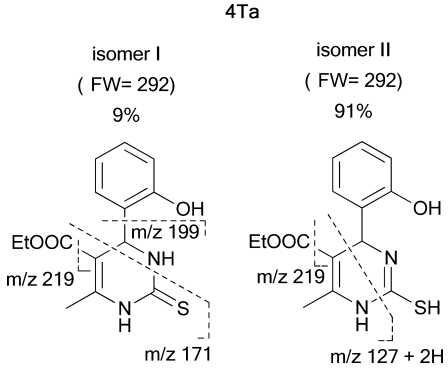

Figure 3. MS fragmentation of isomers **I** and **II** of compound **4Ta**.

imine tautomer **II** appears be stable enough to be isolated even if its separation from tautomer **I** by liquid chromatography (see Supporting Information for the solvent systems) was very hard, thus allowing its characterization always as a mixture with tautomer **I**. In this structure, an intramolecular hydrogen bond probably stabilizes the imine structure **II** giving rise to a six-membered ring (Figure 4) as indicated by the shift of the CH-

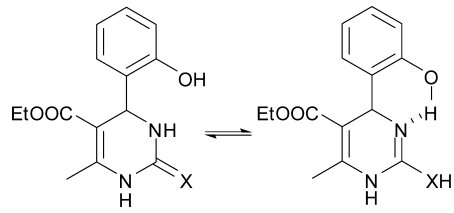

Figure 4. Imine−amide tautomeric equilibrium in compounds **4Ua** and **4Ta**.

proton and the number of quaternary carbon atoms in the ^{1}H NMR and ^{13}C-spectra of the two mixtures of isomers of **4Ua** and **4Ta** (Supporting Information). The much higher percentage of the imine tautomer in this mixture can be explained by the major higher acidity of the thio-analogue compared to **4Ua**.[45] A computational analysis based on density functional theory (DFT) calculations of Monastrol and compounds **4Ua** and **4Ta** indicates lower stability of all the imine and thioimine tautomers with respect to the corresponding amide and thioamide forms. DFT calculations, using several computational protocols and different physical conditions (gas-phase, chloroform, and water solutions), indicate a thermodynamic stabilization of the amide and thioamide forms in the range of 13−22 kcal/mol with respect to their corresponding tautomers (Supporting Information). These results are in agreement with the high stability of the amide forms of Monastrol but do not provide thermodynamic explanation for the formation of the **4Ua**^{II} and **4Ta**^{II} imine tautomers. Notably, the formation of the six-membered ring is strongly favored only in the deprotonated (negatively charged) forms of compounds **4Ua** and **4Ta**. Preliminary DFT studies of the thermodynamic stability of key intermediates along the Biginelli reaction mechanism also support the higher stability of amide and thioamide forms, indicating that thermodynamic effects could not be invoked to justify the formation of the **4Ua**^{II} and **4Ta**^{II} imine tautomers. More detailed computational analysis of the reaction mechanism is needed to evaluate possible kinetic effects associated with the presence of the erbium catalyst. Finally, calculated dipole moments of the tautomeric forms of Monastrol, **4Ua**, and **4Ta** indicate a strong difference (around 4−5 D, see Supporting Information) between the dipole moments of **4Ua**^{I} and **4Ta**^{I} isomers and those of **4Ua**^{II} and **4Ta**^{II} imine tautomers but not in case of Monastrol (with thioamide and thioimine forms having the same dipole moments), in agreement with the observed chromatographic behavior.

CONCLUSIONS

In summary, we present herein an ecofriendly method to obtain a representative range of 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) under solvent-free conditions in a microwave or Q-tube reactor. The yields of DHPMs under these conditions were improved further by the addition of a catalytic amount of erbium trichloride hexahydradate to obtain the products in

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good to excellent yields. As reported in Table 1, the Q-tube technology gave the same results as the MW-assisted protocol but with cleaner reaction profile and in a shorter reaction time, so that it is possible to conclude that in the present example the Q-tube equipment can be proposed as a valid alternative to monomode MW technology in terms of efficiency, safety (virtually eliminating the risk of pressure explosions), and cleanness of the process. Finally, particular attention was devoted to the synthesis of the positional isomers of Monastrol in the aromatic subunit, which were all obtained in good to excellent yields, although it was previously reported that benzaldehyde substituted with a hydroxyl group has not been often used when Lewis acids acted as catalysts, probably because a phenolic hydroxyl group can deactivate the Lewis acid itself.[12]

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, GC/MS-ESI analysis, ^{1}H NMR and ^{13}C NMR spectra, and theoretical calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

$ErCl_3$ is less toxic than table salt; oral mouse LD50 for $ErCl_3$ and NaCl from Sigma−Aldrich security sheets are 4417 and 4000 mg kg−1, respectively. The prices for $ErCl_3$hexahydrate is 288,50 € (for 100 g). Information about Q-tube (prize, applications, validation tests) can be found at the following Web site: http://qlabtech.com/index.html.

The authors declare no competing financial interest.

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