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Analysis by GC-MS of an Aza-Michael Reaction Catalyzed by CALB on an Orbital Shaker and under Microwave Irradiation

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I. Introduction

Chiral amines are an important class of organic compounds, on account of their utility in the preparation of pharmaceutical and industrial products of considerable interest [1,2]. These compounds are intermediates in the synthesis of a large number of organic compounds and are widely used to prepare derivatives of natural products, antibiotics, chiral auxiliaries and lactams. Owing to their vast range of applications, the modification and improvement of techniques for synthesizing *β*-amino carbonyl compounds has been a research objective in recent years [2, 3].

The conjugate addition of the nucleophilic nitrogen in compounds such as amines to *α,β*-unsaturated carbonyl or nitrile compounds, namely the aza-Michael reaction, constitutes a key reaction for the construction of C-N bonds in the preparation of *β*-amino esters and nitriles [4]. This reaction is highly versatile as it can occur between various *N*-nucleophiles (aliphatic and aromatic amines, amides, carbamates and azides) and Michael acceptors (enones, acrylates, unsaturated nitriles, amides, sulfones, phosphonates, trifluoromethylalkenes and nitroalkenes) [5].

Recently, an efficient enzymatic protocol was reported for the synthesis of *β*-amino esters via aza-Michael addition of primary and secondary amines to acrylates; CALB (*Candida antarctica* lipase B) was the biocatalyst and the corresponding *β*-amino esters were produced in good yields [6]. Green chemistry, in which harmful organic solvents and extreme conditions are avoided, is growing in importance; furthermore, the catalyst can be recycled. An efficient and simple protocol for aza-Michael addition of aliphatic and aromatic amines to electron-deficient alkenes has been established, using TMG-based ionic liquids as catalyst under solvent-free conditions [7]. Solvent-free aza-Michael reactions between a variety of amines and *α,β*-unsaturated carbonyl compounds under microwave irradiation, catalyzed by perchloric acid impregnated on silica gel ($HClO_4$=SiO_2), produced the corresponding adducts [8]. Another method has been developed for the aza-Michael addition of acrylonitrile to 2-aryl-oxymethylbenzimidazole derivatives, in the presence of anhydrous potassium carbonate under microwave irradiation, to synthesize a novel series of 1-cyanoethyl-2-aryloxymethylbenzimidazole derivatives [9]. The present paper summarizes the results of our investigation into the conjugate addition of nitrogen-containing nucleophiles to electron-deficient ketones, in which environmentally-friendly enzymatic catalysis was carried out in an orbital shaker and under microwave irradiation, yielding aza-Michael imine adducts and reverse aza-Michael compounds.

II. Materials and Methods

a) General procedure

The Michael addition reactions were carried out on a Tecnal TE-421 orbital shaker. The performance of the enzymatic reactions was measured by analyzing the products in a Shimadzu GC 2010 gas chromatograph equipped with an AOC 20i auto injector, a flame ionization detector (FID) and a J&W Scientific DB-5 column (30 m x 0.25 mm x 0.25 μm). The conditions employed in the gas chromatograph were as follows: carrier gas: nitrogen (60 kPa); injector temperature: 250 °C; injector split ratio: 1:20; detector temperature: 300 °C; initial oven temperature: 80 °C for 2 min; final oven temperature: 250 °C for 3 min; heating rate: 10 °C min^{-1}, and total time of analysis: 22.0 min (Supplementary Information – SI). Gas chromatography–mass spectrometry (GC-MS): a Shimadzu GC2010 Plus

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gas-chromatography system coupled to a mass-selective detector (Shimadzu MS2010plus) was used in the electron ionisation (EI) mode. The GC-MS oven was fitted with a DB-5 fused silica column (J&W Scientific 30 m x 0.25 mm x 0.25 μm). The oven temperature was programmed from 50 °C to 270 °C at a heating rate of 10 °C/min, and a total time of analysis: 32.0 min. The injector and detector temperatures were maintained at 300 °C; injector split ratio was 1:20 and helium was used as the carrier gas at a pressure of 60 kPa. The compounds were identified from the fragmentation ions (70 eV) detected in the GC-MS spectra (Supplementary Information – SI). The structures were confirmed after comparison with Mass Spectral Database (NIST 5.0) and by co-injection of authentic standards. Reactions were purified by column chromatography on silica gel (230–400 mesh) eluted with mixtures of *n*-hexane and EtOAc (8:2). Column effluents were monitored by TLC, using aluminium-backed pre-coated silica gel.

b) Enzyme and chemicals

Novozym 435® (component B of the lipase from *Candida antarctica* immobilized on macroporous poly-acrylate resin, with 10,000 propyl laurate units per gram), was a gift from Novo Nordisk (Curitiba-Paraná, Brazil). Solvents (EtOAc, CH_2Cl_2, *n*-hexane, MeOH, toluene, THF) were commercially available and of analytical grade. Cyclohex-2-en-1-one **1**, 1-phenylmethanamine **2** and 3-methylcyclohex-2-en-1-one **5** were purchased from Sigma-Aldrich and 2,5-dimethyl-*para*-benzoquinone **8** was synthesized from 2,5-dimethyl-phenol [10, 11].

c) Lipase-catalyzed aza-Michael reactions under conventional conditions (orbital shaker)

CALB lipase (80 mg), the unsaturated ketone [2-cyclohexen-1-one **1** (40 μL, 0.41 mmol), 3-methylcyclohex-2-en-1-one **5** (40 μL, 0.35 mmol) or 2,5-dimethyl-*para*-benzoquinone **8** (40 μL, 0.33 mmol)] and the amine [1-phenylmethanamine **2** (40 μL, 0.36 mmol)] were separately added to 10 mL of the organic solvent (*n*-hexane, toluene, dichloromethane, tetrahydrofuran or methyl alcohol) in 50 mL Erlenmeyer flasks. The flasks were sealed with a rubber stopper and the reaction mixture was shaken in an orbital shaker at 33 °C and 133 rpm. The reaction progress was monitored by collecting samples (0.5 mL) at intervals, between 2 and 48 hours from the start, and analyzed by gas chromatography GC-FID and GC-MS. The enzymatic aza-Michael reactions were carried out in triplicate.

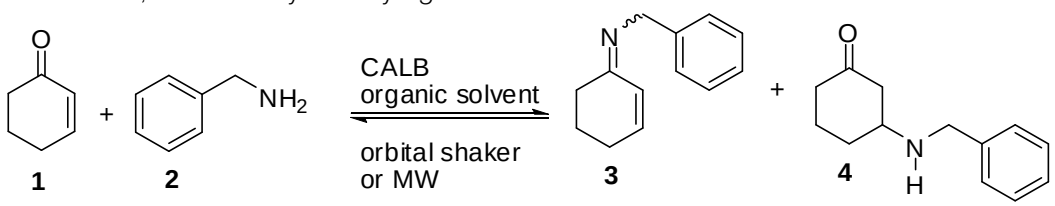
d) Lipase-catalyzed aza-Michael with microwave irradiation

The microwave irradiation (MW) experiments were performed with a Discover System from CEM Corporation. Organic solvents (10 mL, EtOAc, CH_2Cl_2, *n*-hexane, MeOH, toluene, THF), primary amine **2** (40 μL, 0.36 mmol), Michael acceptors **1** (40 μL, 0.41 mmol), **5** (40 μL, 0.35 mmol) and **8** (40 μL, 0.33 mmol) and CALB (80 mg) were separately added to a 50 mL flat bottom flask. The whole reaction mixture was placed in the microwave oven at a frequency 2.45 GHz and irradiated at 33, 40, 50 and 60 °C, power output of about 70 W. The reaction progress was monitored by collecting samples (0.1 mL) and analyzed by gas chromatography with a J & W Scientific DB-5 (30 m x 0.25 mm x 0.25 μm). After 3 hours of reaction, the flask was removed and the immobilized lipase was filtered off. The organic solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with 8:2 hexane and ethyl acetate as eluent. The reactions were carried out in triplicate and the results are presented and discussed next.

III. Results and Discussion

The lipase from *Candida antarctica* (CALB) is often used to promote asymmetric reactions via transesterification of racemic alcohols and amines by chemo-enzymatic resolution [12-15]. The enzymes used to catalyze unusual reactions, commonly named promiscuous enzymes, such as the Michael addition reaction catalyzed by lipases, are being investigated with interest [6, 16-17].

The aim of this study was to carry out Michael additions with the immobilized lipase from *C. antarctica*, on account of its proven catalytic efficiency and thermal stability, with high activity at 70-90 °C [18]. Our study began with cyclohex-2-enone **1**, due to the absence of steric hindrance in the 1,4-addition, and the achiral primary amine, 1-phenylmethanamine **2**. Under these conditions, the reaction can favor two products, the imine *N*-(cyclohex-2-en-1-ylidene)-1-phenylmethanaminne **3** and the chiral aza-Michael adduct 3-(benzylamino) cyclohexanone **4** (Scheme 1).



Scheme 1 : Aza-Michael reactions using CALB on an orbital shaker and microwave irradiation

Initially, this reaction was tested in five organic solvents (*n*-hexane, dichloromethane, methanol, tetrahydrofuran and toluene). It was also performed in the absence of CALB, since primary amine **2** is a strong nucleophile and *α,β*-unsaturated ketone **1** is a good Michael acceptor, which could favor the spontaneous formation of products **3** and **4**.

Under orbital shaking, in the presence of CALB, this reaction gave the aza-Michael adduct **4** at low conversion rates, varying with the solvent used (c 9-37%), and imine **3** at conversions of 5-23%, in 48 h. The non-enzymatic reaction gave similar results for adduct **4** (c 3-29%) and imine **3** (4-34%) in 48 h. The reaction in the presence of CALB and *n*-hexane produced adduct **4** at 25% conversion and imine **3** at 23% in 48 h, while under the same conditions without CALB lower yields of both adduct **4** (c 10%) and imine **3** (c 9%) in 48 h were observed. In this case, there is a visible influence of lipase in the formation of the aza-Michael adduct **4** (SI-1). In methanol, there was good conversion to the adduct **4** (c 38%) and imine **3** (c 14%) in 48 h. In the absence of CALB, a decrease occurred in the conversion of adduct **4** (c 29%) and an increase in conversion to imine **3** (c 35%) in 48 h (SI-2).

It is reported in the literature that CALB exhibits good catalytic activity in hydrophobic solvents [12, 14, 19]. In our studies the reactions in *n*-hexane and methanol were not efficient. Factors such as the polarity of the substrates (ketone **1** and amine **2**) may have influenced these results, and Michael addition reactions may be occurring spontaneously and reversibly, yielding unstable products, without being promoted by CALB [17].

In dichloromethane, with CALB, the reaction showed low conversion to adduct **4** (c 10%) and imine **3** (c 6%) after 48 h. In the absence of lipase we observed similar values to those obtained enzymatically for adduct **4** (c 7%) and imine **3** (c 4%), at 48 h (SI-3).

In toluene, adduct **4** showed low conversion (c <14%), as did imine **3** (c 11%), after 48 h. In the non-enzymatic reaction, adduct **4** was obtained at a conversion of less than 3% and the imine at 2% in 48 h (SI-4). The choice of solvent depends strongly on the solubility of the catalyst and of the donor and acceptor in the Michael addition, as well as the ability of the solvent to prevent the occurrence of side reactions. For example, if the reactant or products are susceptible to alcoholysis (ester hydrolysis), transesterification, autocondensation, reverse Michael reactions, non-hydroxyl solvents are desirable [20].

Aiming to improve the conversion of aza-Michael addition in the presence of CALB, the reactions with ketone **1** and amine **2** were promoted, in methanol, THF, dichloromethane and *n*-hexane, by microwave irradiation (40 °C, 70 W). Under these conditions, the aza-Michael adduct **4** showed 0.3-42% conversion and imine **3** of 2-40% conversion, depending on the solvent used. The reaction in methanol gave the best result, the adduct **4** was produced at 42% conversion and imine **3** at 4%, in 3 h.

In *n*-hexane and dichloromethane, only imine **3** (c 12%) was formed in 3 h. In THF, there were low conversions of adduct **4** (0.3-6%) and imine **3** (1-3%) in 3 h. The reactions in dichloromethane and hexane, without CALB, yielded the adduct **4** and imine **3** in small amounts (c<10%) by microwave irradiation for 3 h.

The regioselectivity of the reactions depends on the conditions in which they are carried out, the type of catalyst, the solvent and the nucleophile [21]. In these studies, the reaction both in the presence and absence of CALB favored the formation of products via 1,2-addition and 1,4-addition with similar conversions.

Dhake et al. (2010) conducted aza-Michael additions between various primary and secondary amines and acrylates, in the presence of lipase, to obtain the corresponding *β*-amino esters. In this study, methyl acrylate and CALB in toluene showed the best results in the synthesis of the Michael adduct. It was observed that the activity of the enzyme increased with the temperature, up to 60 °C. However, at higher temperatures (> 80 °C), the yield of the reaction decreased to 75%, possibly due to inactivation of the enzyme [6].

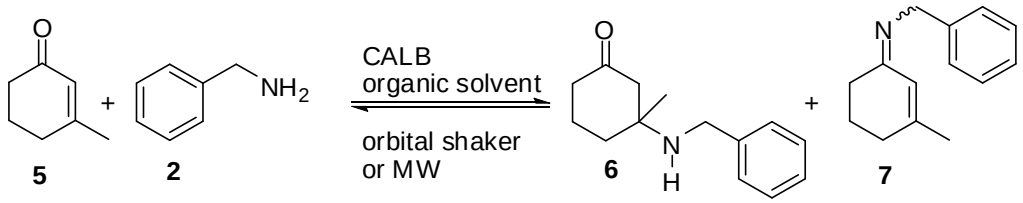
All the reactions in this first part were monitored by GC-FID and GC-MS analysis, which showed the formation of the aza-Michael adduct **4** and imine **3** (Supplementary Information - SI). During the isolation of the adduct **4** by silica gel only the starting reactants, namely enone **1** and the amine **2**, were found. Thus, it was concluded that a reverse Michael reaction occurred during the purification process. The instability of these products was confirmed by the main fragments obtained in the mass spectra, which were compared with data from the Nist 5.0 library.

In the GC-MS chromatogram of the reaction, in *n*-hexane under orbital shaking for 4 days (SI-5), a signal with a retention time of 14.5 minutes was observed, which provided the spectrum of a molecular ion at *m/z* 185 $[M]^+$. The mass spectrum of imine **3** was comparable to the Nist 5.0 mass spectral database. The fragmentation of the molecular ion at *m/z* 185 provided the tropylium ion, at *m/z* 91, as the base peak. On the basis of these fragmentations it was possible to suggest the formation of imine **3** (SI-5 and SI-6).

Another signal in the chromatogram, with retention time 15.9 minutes, showed the molecular ion at *m/z* 203 that corresponds exactly to the aza-Michael adduct **4**. The mass spectrum was similar to that reported for adduct **4** in the Nist 5.0 mass spectral database, and is consistent with the literature [22, 23]. The fragmentation pattern of the molecular ion at *m/z* 203 again provided the tropylium ion (*m/z* 91) as a base peak, which confirmed the formation of adduct **4** (SI-5 and SI-6).

Thus, we conclude from the spectral evidence that it is possible to obtain the compounds **3** and **4** from enone **1** and amine **2**. However, due to the instability of the products, they were not isolated, but were characterized by mass spectrometry.

The next step was to evaluate the regioselectivity of the Michael reaction by using the substituted acceptor, 3-methylcyclohex-2-en-1-one **5** and the achiral donor, 1-phenylmethanamine **2**. The reactions were performed in the presence and absence of CALB in the orbital shaker, with hexane and methanol, and under microwave irradiation, with methanol, dichloromethane and hexane (Scheme 2).



Scheme 2 : Aza-Michael reactions using CALB on an orbital shaker and microwave irradiation

The reactions under orbital shaking were similar, with or without CALB, in methanol: the imine **7** was produced at 27-38% conversion in 24 h, whereas the adduct **6** was not detected under these conditions (SI-7).

In hexane and with CALB, adduct **6** was not observed after 24 h, but only imine **7** at 30% conversion (SI-8). In the absence of lipase, imine **7** was produced at less than 5%. In this case, CALB influnced greatly the product obtained by 1,2-addition. In these reactions, the methyl group at position 3 of enone **5** hinders the Michael 1,4-addition, probably by steric and electronic effects. The larger the substituent group on the ***α,β***-unsaturated ketone, the more unfavorable is the 1,4-addition [20].

Initially, good yields were expected for adduct **6** with lipase in hexane, because the enzyme acts very well in nonpolar solvents. However, ketone **5** and amine **2** have polar groups that may influence the low conversion to imine **7**.

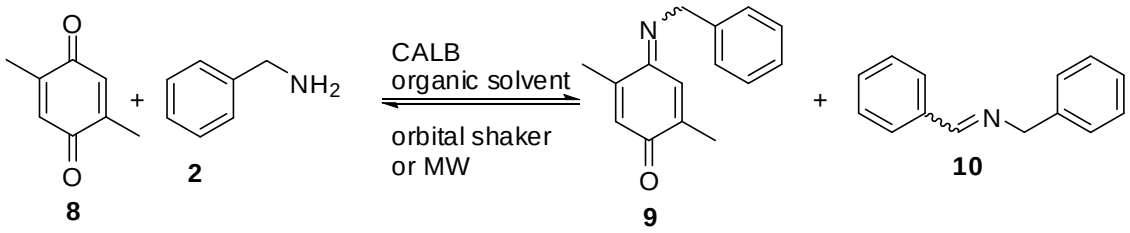
The reactions under microwave irradiation were performed at 33 and 50 °C and 70 W. The enzymatic reaction in methanol and dichloromethane at 33 °C did not form adduct **6** and imine **7** showed conversion < 5%.

Yu et al. studied the effects of temperature on the CALB in the transesterification of (±)-2-octanol by microwave irradiation. In that study the temperature was assessed from 40 to 100 °C, and the activity of the enzyme increased with temperature up to 80 °C, while there was a fall in enzymatic activity at higher temperatures [24]. The elevated temperature in a microwave reactor increases the probability of collision between enzyme and substrate molecules, which can promote the formation of the enzyme-substrate complex and increase the rate of reaction and the final conversion [24].

Given the low conversion of reaction in methanol and dichloromethane, the temperature was raised to 50 °C and 70 W, resulting in a better performance of the reaction in methanol in the formation of the ***α,β***-unsaturated imine **7**, but with low conversion (c = 10%). The results of these experiments were similar in the enzymatic and non-enzymatic reactions (c<11%, 50 °C, 70 W), yielding only the imine **7** at low conversion. There are several factors that may influence the low performance of these reactions. Amine **2** may be interacting with other parts of the lipase, hampering the Michael addition under these conditions. Also these reactions are subject to the reverse aza-Michael pathway; thus, the instability of imine **7** was confirmed by the main fragments obtained in the mass spectra by GC-MS, compared with data from the Nist 5.0 library (SI-8).

Analysis of the chromatogram of the reaction performed in hexane by orbital shaking, showed the presence of a signal with a retention time of 15.9 minutes. Its mass spectrum showed the ion fragment at *m/z* 199, corresponding to imine **7** (SI-8 and SI-9). To confirm the formation of imine **7**, the reaction was performed in the orbital shaker with methanol. After the reaction was complete and the presence of imine **7** confirmed by GC-MS, 10 mL of distilled water was added and the reaction was maintained under shaking for one hour. After extraction, the reaction was analyzed by GC-MS and showed the reacting species **5** and **2**.

Finally, we carried out the reaction with primary amine **2** and 2,5-dimethyl-*para*-benzoquinone **8**. The experiments were conducted in hexane with CALB on an orbital shaker and under microwave irradiation for 96 h. After this time, two products with retention times of 15.0 minutes (c 43%) and 19.0 minutes (c 34%) were detected by GC-MS analysis (Scheme 3) (SI-10).



Scheme 3 : Aza-Michael reactions using CALB on an orbital shaker and under microwave irradiation

The reactions were conducted in hexane and microwave reactor at 60 °C (70 W) in the presence of CALB. After 5h, the reactions yielded two products with retention times of 15.0 minutes (c 21%) and 19.0 minutes (c 18%) with complete consumption of amine **2** by GC-MS analysis (SI-11).

Analysis of the mass spectrum of the compound with retention time 15.0 minutes showed the presence of a molecular ion with the base peak at *m/z* 225. There was an intense peak at *m/z* 148 (75%) corresponding to the loss of a fragment at *m/z* 77. These fragments are consistent with the structure of the compound 4-(benzylimino)-2,5-dimethylcyclohexa-2,5-dienone **9** (SI-11 and SI-12).

The compound with retention time 19.0 minutes provided a mass spectrum for a molecular ion at *m/z* 195 and base peak at *m/z* 91. Analysis of the mass spectra with reference to the Nist 5.0 library and in comparison with the literature [25] suggested the formation of *N*-benzylidene-1-phenylmethanamine **10** (SI-11 and SI-12).

The formation of **10** could occur by nucleophilic attack of amine **2** on imine **9**. The non-enzymatic reaction does not occur under microwave irradiation at 3 h. However, the reaction under microwave irradiation promoted the formation **10** in face of the reactivity of the species involved (SI-11 and SI-12).

Finally, to confirm the production of imines **9** and **10**, the reaction mixture was subjected to hydrolysis at 24 hours, then extracted with ethyl acetate and analyzed by GC-MS. After hydrolysis, products **9** and **10** were all converted to the starting reagents **2** and **8**, confirming the synthesis of unstable imines in aqueous medium (SI-13 and SI-14).

In the chromatogram of the hydrolysis reaction, no amine **2** was observed, but the corresponding *N*-benzylacetamide **11** with retention time 11.3 minutes was formed by acetylation of amine **2** during extraction with ethyl acetate. The compound was confirmed by synthesis of the authentic standard (SI-15).

IV. Conclusion

In this paper we present studies on the regioselectivity of aza-Michael addition reactions between amine **2**, and enones **1**, **5** and **8** under various experimental conditions, such as types of organic solvent, presence and absence of lipase from *Candida antarctica*, orbital shaking and microwave irradiation. All the reactions yielded products via the formation of 1,2- and 1,4-additions. Owing to the poor stability of the compounds formed, GC-MS analyzes were carried out and showed the formation of aza-Michael adducts and several imines. Reverse aza-Michael reactions and hydrolysis of imines allowed us to verify the formation of all the products. In some cases, it was also possible to observe the influence of lipase and microwave irradiation on the performance of the reactions. The compounds were confirmed by detailed analysis of their mass spectra and the corresponding ions formed.

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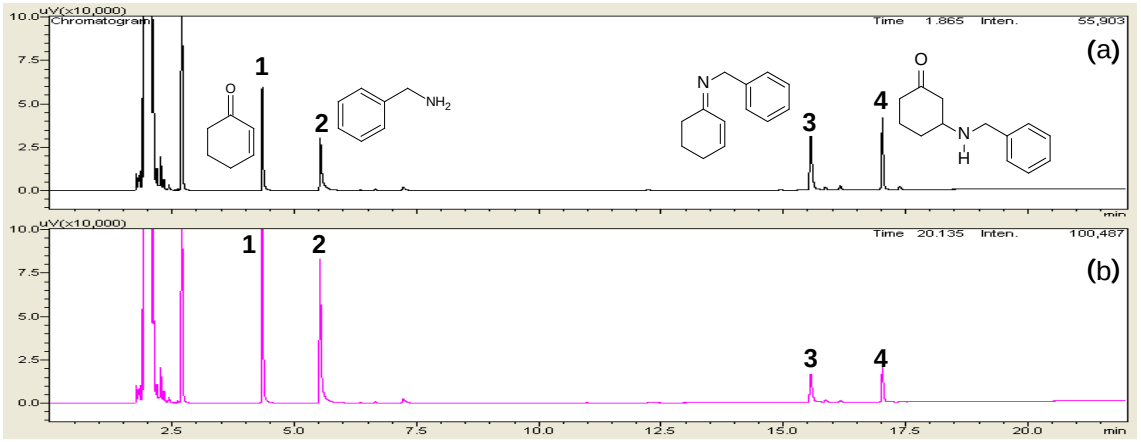
References Références Referencias

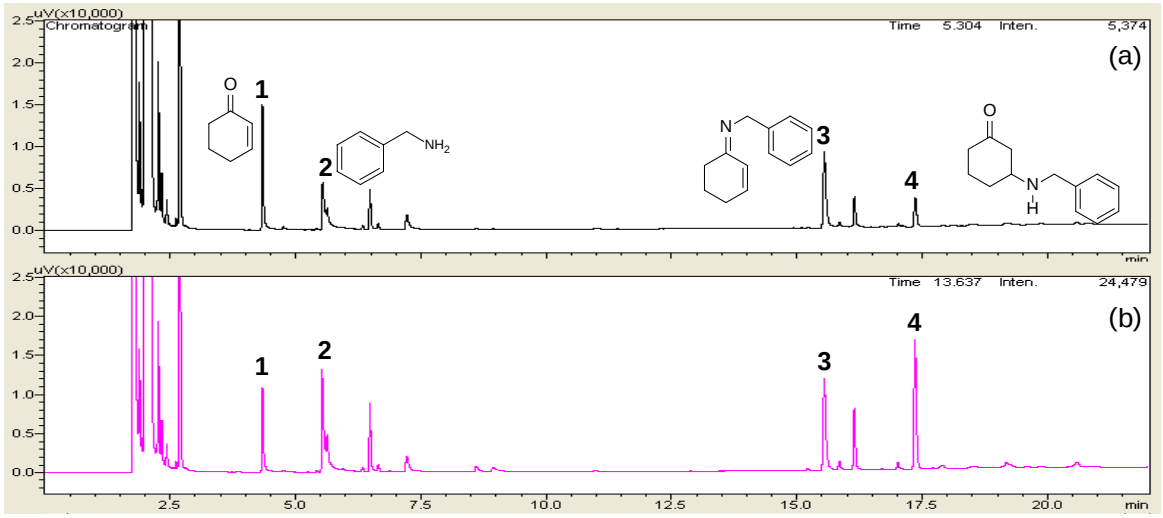
1. Gotor-Fernández, V.; Busto, E.; Gotor, V. *Candida antarctica* lipase B: An ideal biocatalyst for the preparation of nitrogenated organic compounds. Adv. Synth. Catal. 2006; 348: 797-812.
2. Yadav, J. S.; Reddy, B. V. S.; Basak, A. K.; Narsaiah, A. V. Aza-Michael reactions in ionic liquids. A facile synthesis of α-amino compounds. Chem. Lett. 2003; 32: 988-989.
3. Zhang, H.; Zhang, Y.; Liua, L.; Xua, H.; Wang, Y. $RuCl_3$ in poly(ethylene glycol): A highly efficient and recyclable catalyst for the conjugate addition of nitrogen and sulfur nucleophiles. Synthesis. 2005; 13: 2129–2136.
4. Trivedi, R.; Lalitha, P.; Roy, S. Vanadyl(IV) acetate: An efficient, reusable heterogenous catalyst for aza-Michael reaction under solvent-free conditions. Synth. Commun. 2008; 38: 3556-3566.
5. Rulev, A. Y. Aza-Michael reaction: achievements and prospects. Russian Chem. Rev. 2011; 80: 197-218.

6. Dhake, K. P.; Tambade, P. J.; Singhal, R. S.; Bhanage, B. M. Promiscuous *Candida antarctica* lipase B-catalyzed synthesis of *β*-amino esters via aza-Michael addition of amines to acrylates. Tetrahedron Lett. 2010; 51: 4455–4458.
7. Ying, A.; Zheng, M.; Xu, H.; Qiu, F.; Ge, C. Guanidine-based task-specific ionic liquids as catalysts for aza-Michael addition under solvent-free conditions. Res. Chem. Intermed. 2011; 37: 883–890.
8. Singh, S. P.; Kumar, T. V.; Chandrasekharam, M.; Giribabu, L.; Reddy, P. Y. Microwave-assisted, rapid, solvent-free aza-Michael reaction by perchloric acid impregnated on silica gel. Synth. Commun. 2009; 39: 3982–3989.
9. Wei, T-B.; Hua, M-T.; Shi, H-X.; Liu, Y.; Zhang, Y-M. Aza-Michael addition of acrylonitrile with 2-aryloxymethylbenzimidazole derivatives under microwave irradiation. J. Chem. Research. 2010; 34: 452-454.
10. Dockal, E. R.; Cass, Q. B.; Brocksom, T. J.; Brocksom, U.; Corrêa, A. G. Synth. A simple and efficient synthesis of thymoquinone and methyl *p*-benzoquinone. Commun. 1985; 15: 1033-1036.
11. Uliana, M. P.; Vieira, Y. W.; Donatoni, M. C.; Corrêa, A. G.; Brocksom, U.; Brocksom, T. J. J. Braz. Chem. Soc. Oxidation of mono-phenols to *para*-benzoquinones: a comparative study. 2008; 19: 1484-1489.
12. Ribeiro, S. S.; Raminelli, C.; Porto, A. L. M. Enzymatic resolution by CALB of organofluorine compounds under conventional condition and microwave irradiation. J. Fluorine Chem. 2013; 154: 53-59.
13. Ribeiro, S. S.; De Oliveira, J. R.; Porto, A. L. M. Lipase-catalyzed kinetic resolution of (±)-mandelonitrile under conventional condition and microwave irradiation. J. Braz. Chem. Soc. 2012; 23: 1395-1399.
14. Melgar, G. Z.; Wendler, E. P.; Santos, A. A. dos; Porto, A. L. M. First stereocontrolled acetylation of a hydroxypropargylpiperidone by lipase CALB. Tetrahedron: Asymmetry, 2010; 21: 2271–2274.
15. Ferraz, H. M. C.; Bianco, G. G.; Teixeira, C. C.; Andrade, L. H.; Porto, A. L. M. Enzymatic resolution of α-tetralols by CALB-catalyzed acetylation. Tetrahedron: Asymmetry 2007; 18: 1070–1076.
16. Souza, R. O. M. A. de; Matos, L. M. C.; Gonçalves, K. M.; Costa, I. C. R.; Nabics, I.; Leite, S. G. F.; Oestreicher, E. G.; Antunes, O. A. C. Michael additions of primary and secondary amines to acrylonitrile catalyzed by lipases. Tetrahedron Lett. 2009; 50: 2017-2018.
17. Priego, J.; Ortíz-Nava, C.; Carrillo-Morales, M.; López-Munguía, A. Escalate, J.; Castillo, E. Solvent engineering: an effective tool to direct chemoselectivity in a lipase-catalyzed Michael addition. Tetrahedron, 2009; 65: 536-539.
18. Bachu, P.; Gibson, J. S.; Sperry, J.; Brimble, M. A. The influence of microwave irradiation on lipase-catalyzed kinetic resolution of racemic secondary alcohols. Tetrahedron: Asymmetry 2007; 18: 1618-1624.
19. Rocha, L. C.; Rosset, I. G.; Luiz, R. F.; Raminelli, C.; Porto, A. L. M. Kinetic resolution of iodophenylethanols by *Candida antarctica* lipase and their application for the synthesis of chiral biphenyl compounds. Tetrahedron: Asymmetry 2010; 21: 926–929.
20. Mather, B. D.; Viswanathan, K.; Miller, K. M.; Long, T. E. Michael addition reactions in macromolecular design for emerging technologies. Prog. Polym. Sci. 2006; 31: 487-531.
21. Hoz, A. de L.; Días-Ortiz, A.; Gómez, M. V.; Mayoral, J. A.; Moreno, A.; Sánchez-Migallón, A. M.; Vazquez, E. Preparation of α- and β-substitutend alanine derivatives by α-amidoalkylation or Michael addition reactions under heterogeneous catalysis assisted by microwave irradiation. Tetrahedron 2001; 57: 5421-5428.
22. WSS: Spectral data were obtained from Wiley Subscription Services, Inc. (US).
23. Zhang, H.; Zhang, Y.; Liu, L.; Xu, H.; Wang, Y. $RuCl_3$ in poly(ethylene glycol): A highly efficient and recyclable catalyst for the conjugate addition of nitrogen and sulfur nucleophiles. Synthesis 2005; 13: 2129-2136.
24. Yu, D.; Wang, Z.; Chen, P.; Jin, L.; Vheng, Y.; Zhou, J.; Cao, S. Microwave-assisted resolution of (*R,S*)-2-octanol by enzymatic transesferification. J. Mol. Catal. B: Enzym. 2007; 48: 51-57.
25. Montalvo-González, R.; Ariza-Castolo, A. Molecular structure of di-aryl-aldimines by multinuclear magnetic resonance and X-ray diffraction. J. Mol. Structure. 2003; 655: 375-389.

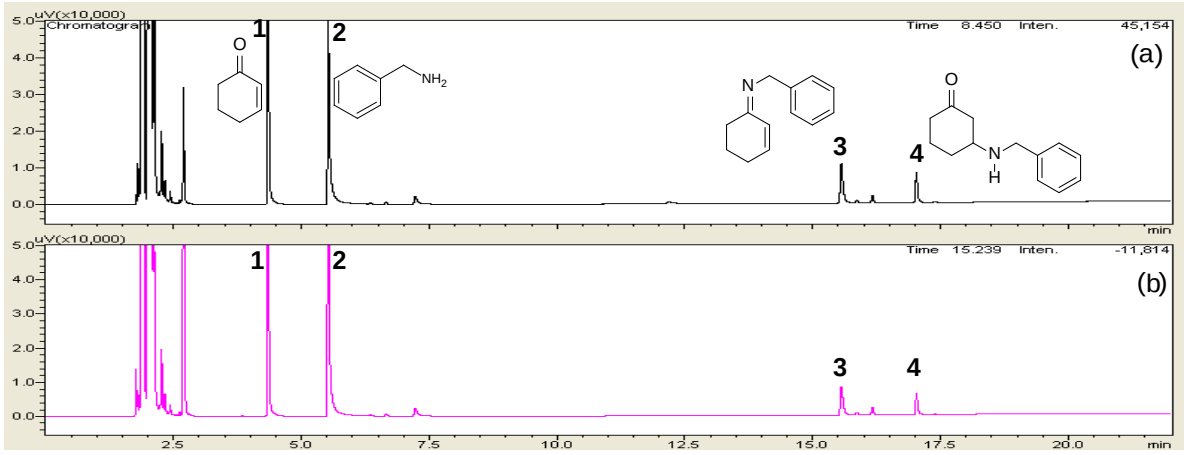
Supplementary Information



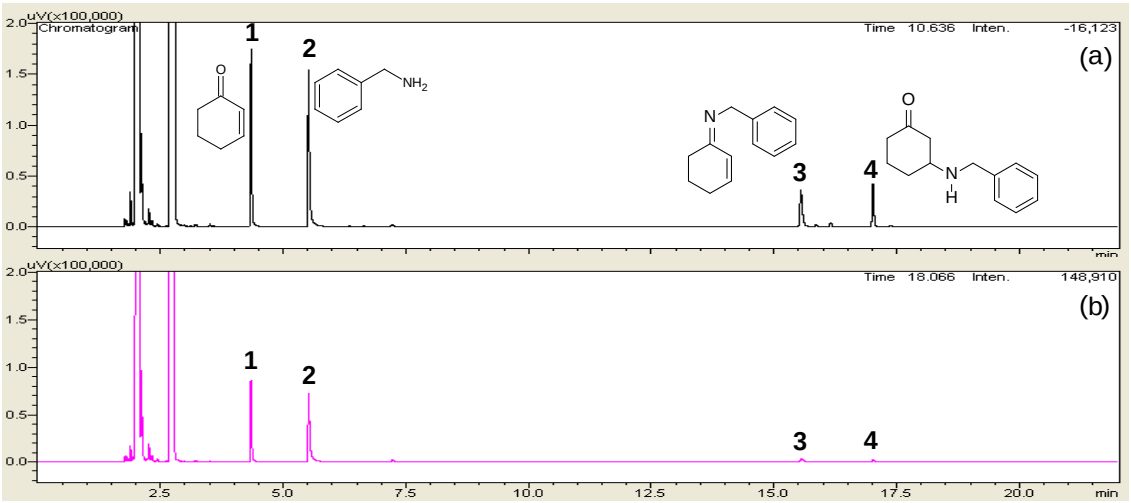
SI-1 : Chromatograms obtained by GC-FID. Reaction of ketone **1** with amine **2** in hexane under orbital shaking (48 h, 33 °C, 133 rpm). (a) In the presence of lipase. (b) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



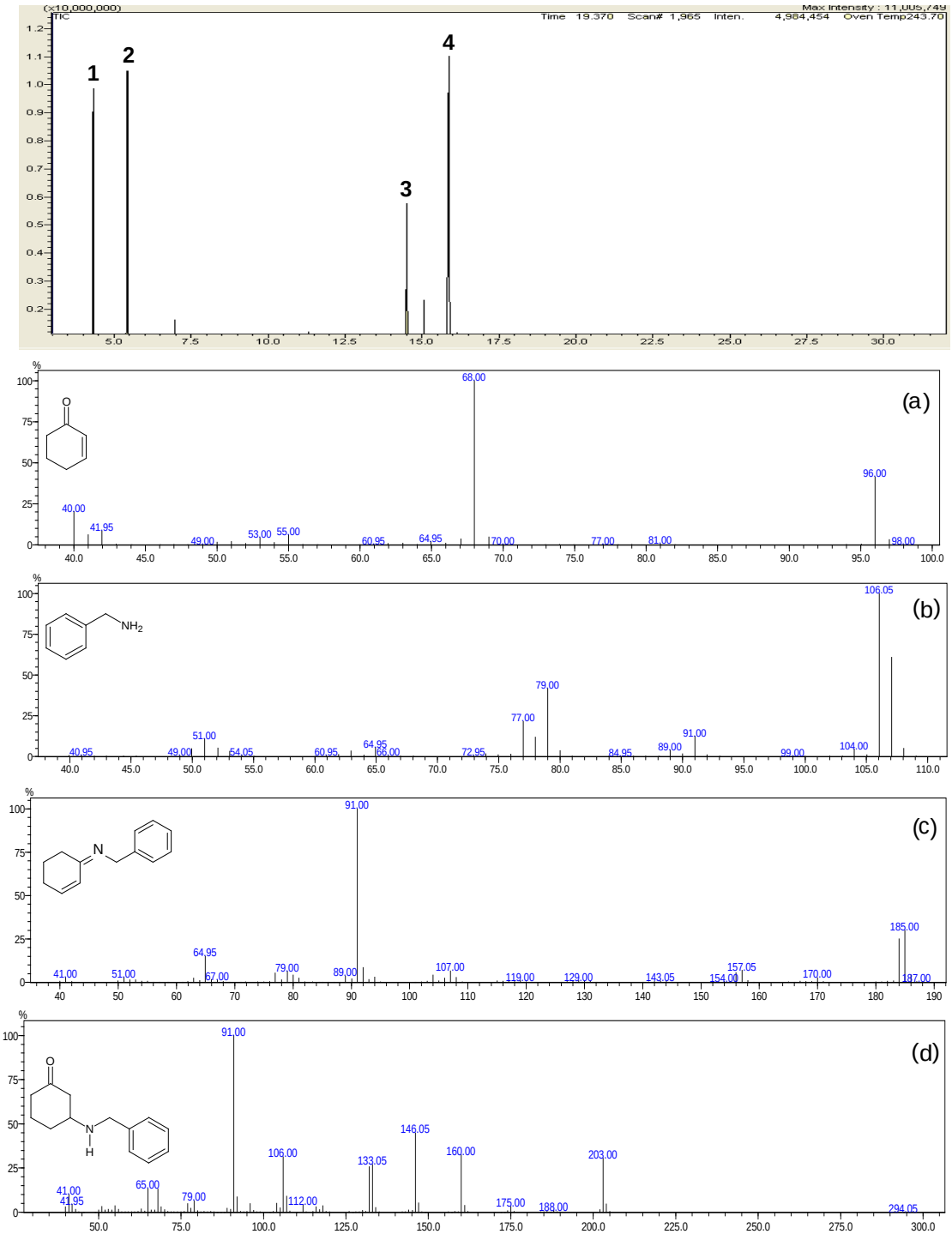
SI- 2 : Chromatograms obtained by GC-FID. Reaction of ketone **1** with amine **2** in methanol under orbital shaking (48 h, 33 °C, 133 rpm). (b) In the presence of lipase. (b) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



SI- 3 : Chromatograms obtained by GC-FID. Reaction of ketone **1** with amine **2** in dichloromethane under orbital shaking (48 h, 33 °C, 133 rpm). (a) In the presence of lipase. (b) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



SI- 4 : Chromatograms obtained by GC-FID. Reaction of ketone **1** with amine **2** in toluene under orbital shaking (48 h, 33 °C, 133 rpm). (b) In the presence of lipase. (b) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



SI- 5 : Chromatogram and mass spectrum obtained by GC-MS (EI, 70 eV) for the reaction of ketone **1** with amine **2** in hexane under orbital shaking for 4 days (33 °C, 133 rpm). (a) Ketone **1**. (b) Amine **2**. (c) Mass spectrum obtained from a signal with a retention time of 14.5 min. (d) Mass spectrum obtained from a signal with a retention time of 15.9 min. Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 μm) Ti = 50 °C, Tf = 270 °C, r = 10 °C/min, t = 32.0 min

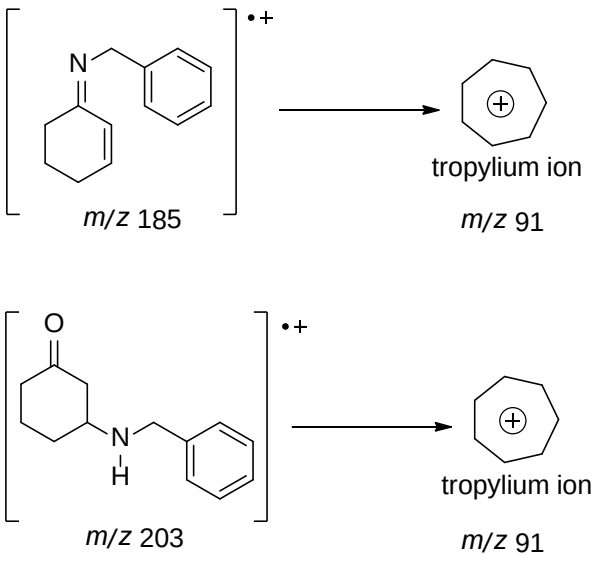
GC-MS (EI, 70 eV):

2-cyclohexen-1-one **1**, C_6H_8O, *m/z* (%): 68 (100), 96 (M^+, 41), 40 (21), 55 (6), 81 (0.5).

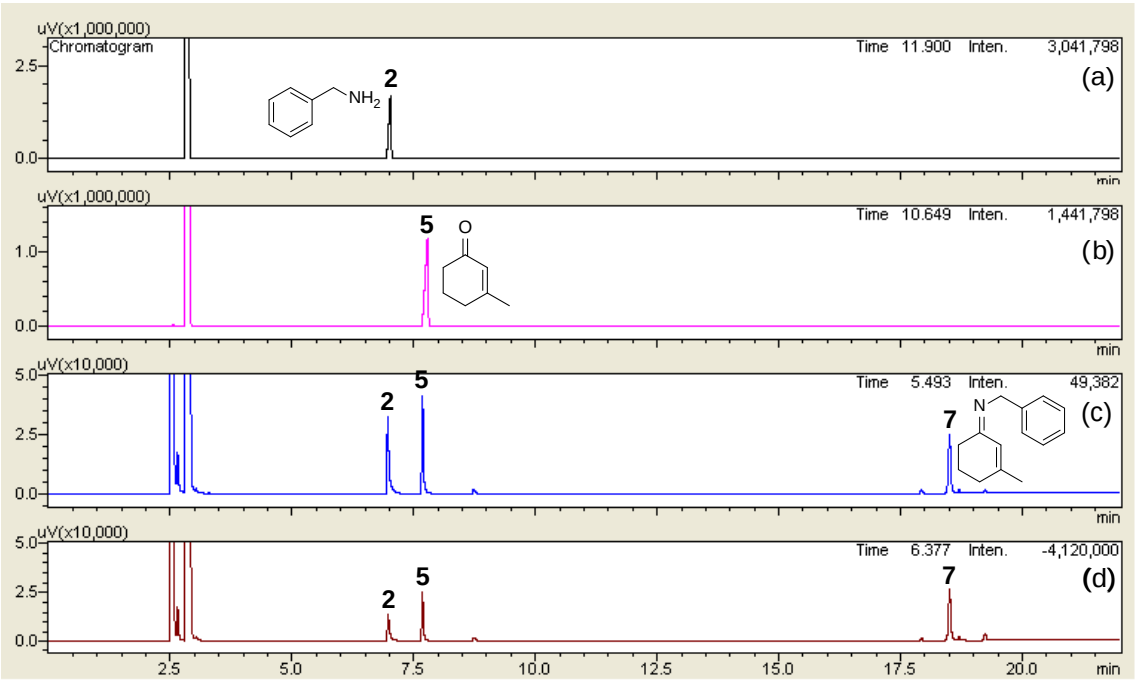
1-phenylmethanamine **2**, C_7H_9N, *m/z* (%): 106 (M^+-1, 100), 107 (M^+, 61), 79 (42), 91(12), 51 (11), 65 (6).

N-(cyclohex-2-en-1-ylidene)-1-phenylmethanamine **3**, $C_{13}H_{15}N$, *m/z* (%): 65 (15), 91 (100), 185 (M^+, 30), 157 (7).

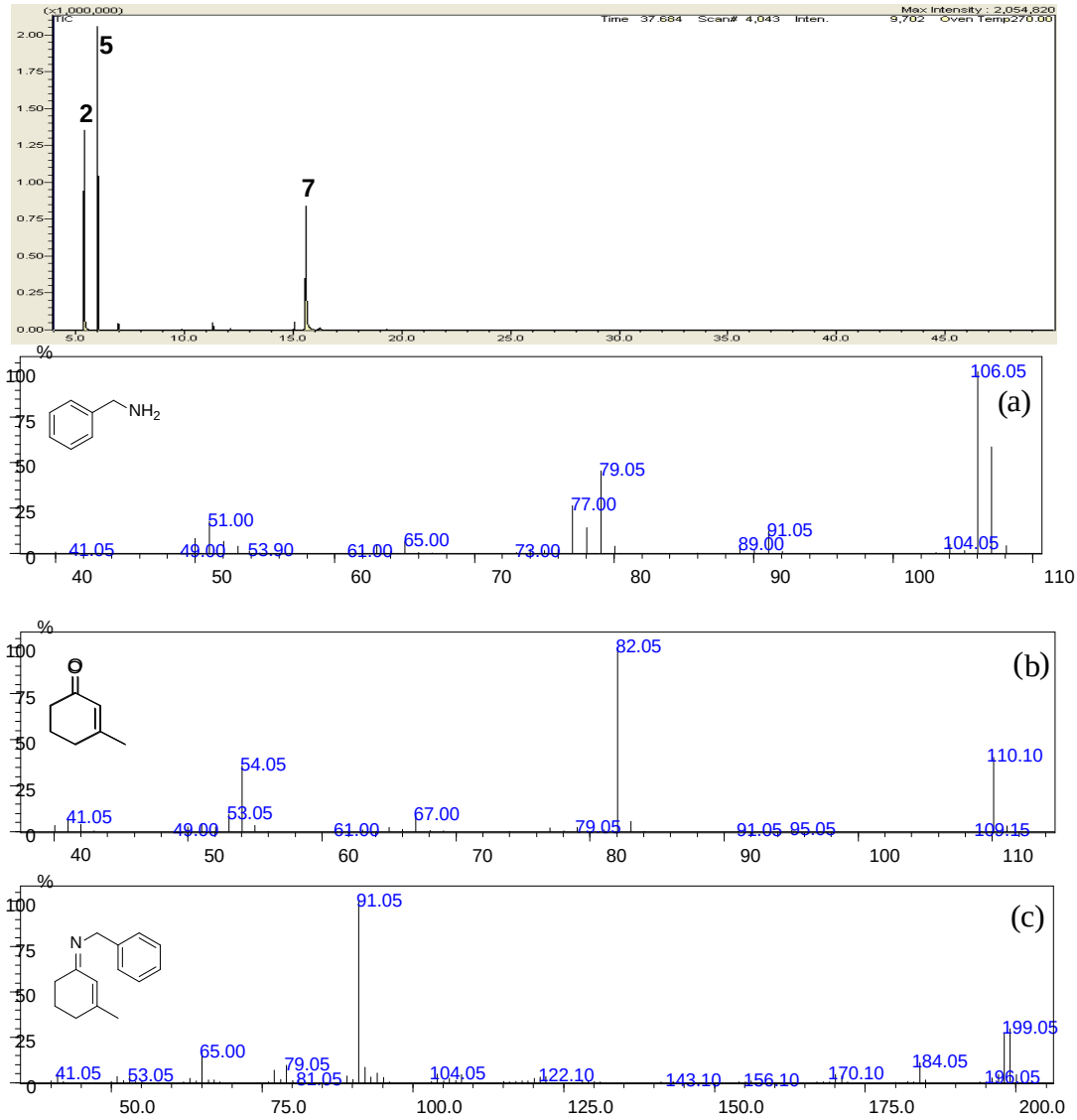
3-(benzylamino)cyclohexanone **4** $C_{13}H_{17}NO$, *m/z* (%): 91 (100), 146 (44), 160 (32), 203 (M^+, 30), 133 (27), 106 (31), 65 (13).



SI-6 : Cleavage probable at the bond β to the ring, giving the tropylium ion



SI- 7 : Chromatograms obtained by GC-FID. (a) Standard of amine **2**. (b) Standard of ketone **5**. (c) Reaction of amine **2** with ketone **5** in methanol under orbital shaking in the presence of lipase (24 h, 33 °C, 133 rpm). (d) In the absence of lipase. Conditions of GC-FID analysis: DB-5 column (30 m x 0.25 mm x 0.25 μm) Ti = 80 °C, Tf = 250 °C, r = 10 °C/min, t = 22.0 min



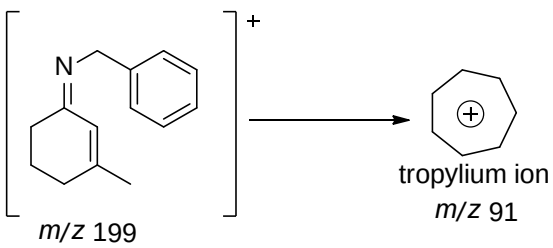
SI- 8 : Chromatogram and mass spectrum obtained by GC-MS (EI, 70 eV) for the reaction of ketone **5** with amine **2** in hexane under orbital shaking (24 h, 33 °C, 133 rpm). (a) Amine **2**. (b) Ketone **5**. (c) Mass spectrum obtained from a signal with a retention time of 15.9 min. Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 μm) Ti = 50 °C, Tf = 270 °C, r = 10 °C/min, t = 32.0 min

GC-MS (EI, 70 eV):

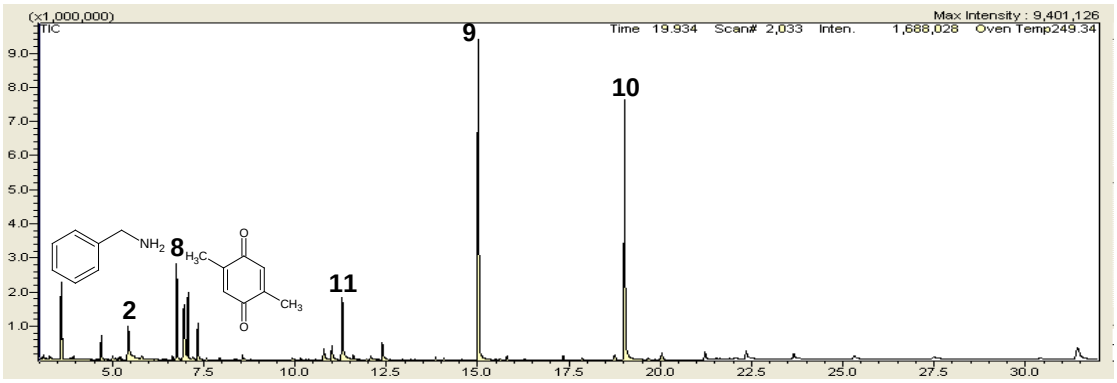
3-methylcyclohex-2-en-1-one **5**, $C_7H_{10}O$, *m/z* (%): 82(100), 110(40, M^+), 54(36), 41(8).

1-phenylmethanamine **2** C_7H_9N, *m/z* (%): 106 (M^+-1, 100), 107 (M^+, 61), 79 (42), 91(12), 51(11), 65 (6).

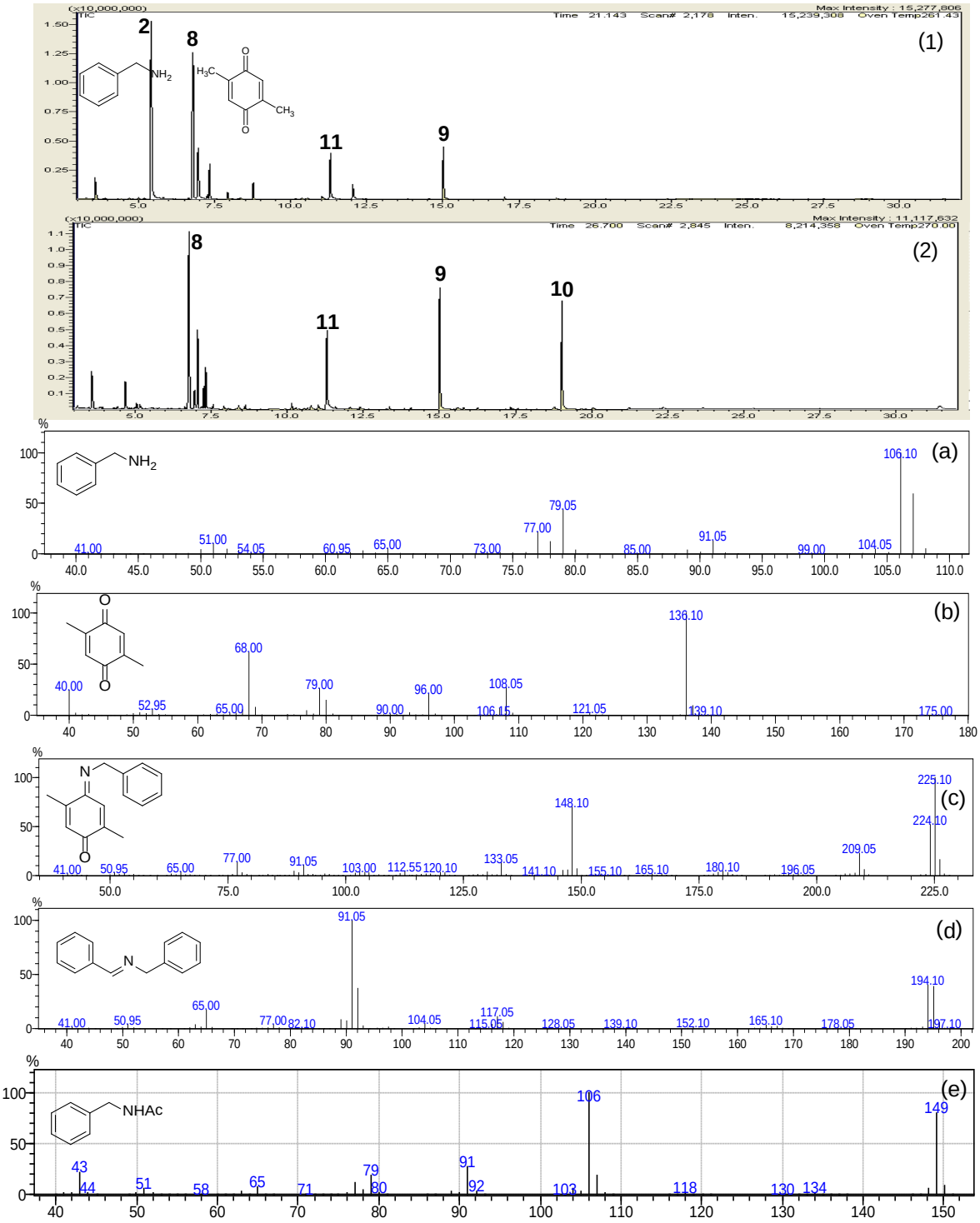
N-(3-methylcyclohex-2-en-1-ylidene)-1-phenylmethanamine **7**, $C_{14}H_{17}N$, *m/z* (%): 91(100), 199 (M^+, 30), 65(17), 184(11), 79(10).



SI-9 : Cleavage probable at the bond β to the ring, giving the tropylium ion



SI-10 : Chromatogram obtained by GC-MS. Reaction of amine **2** with ketone **8** in hexane under orbital shaking (96 h, 33 ^{o}C, 130 rpm). Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 μm) Ti = 50 ^{o}C, Tf = 270 ^{o}C, r = 10 ^{o}C/min, t = 22.0 min



SI-11 : Chromatograms and mass spectrum obtained by GC-MS (EI, 70 eV). (1) Non-enzymatic reaction of amine **2** with ketone **8** in hexane under microwave irradiation (3 h, 60 °C, 70 W). (2) Enzymatic reaction of amine **2** with ketone **8** in hexane under microwave irradiation (5 h, 60 °C, 70 W). (a) Amine **2**. (b) Ketone **8**. (c) Mass spectrum obtained from a signal with a retention time of 15.0 min. (d) Mass spectrum obtained from a signal with a retention time of 19.0 min. (e) Mass spectrum obtained from a signal with a retention time of 11.3 min. Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 μm) Ti = 50 °C, Tf = 270 °C, r = 10 °C/min, t = 32.0 min

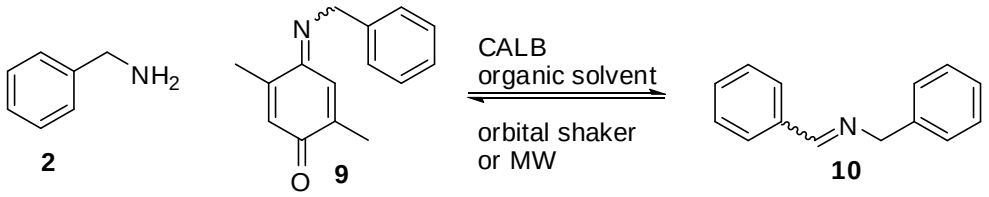
GC-MS (EI, 70 eV):

2,5-dimethyl-*para*-benzoquinone **8**, $C_8H_8O_2$, *m/z* (%): 136(M^+, 100), 68(66), 79(28), 108(29), 40(28), 96(22), 121(3).

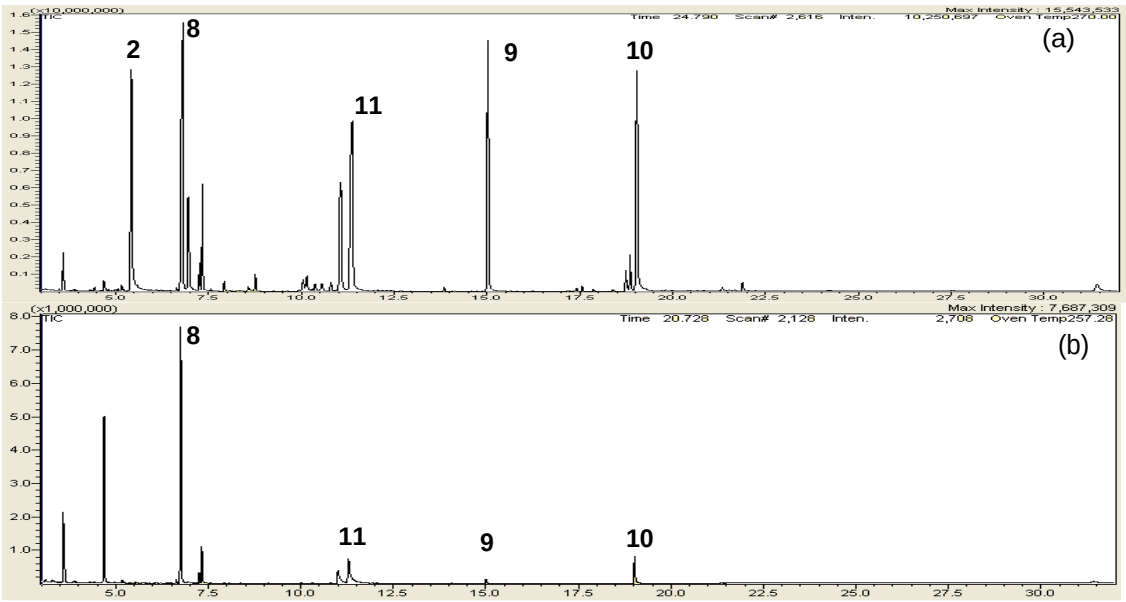
4-(benzylimino)-2,5-dimethylcyclohexa-2,5-dienone **9**, $C_{15}H_{15}NO$, *m/z* (%): 225(M^+, 100), 148(70), 224(52), 209(23), 77(14).

N-benzylidene-1-phenylmethanamine **10**, $C_{14}H_{13}N$, *m/z* (%): 65 (17), 91(100), 92 (37), 117 (11), 194 (40), 195 (M^+, 38).

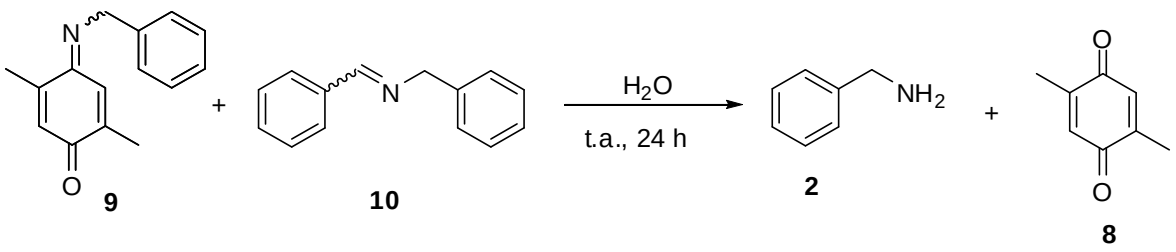
N-benzylacetamide **11** $C_9H_{11}NO$, *m/z* (%): 106 (100), 149 (M^+, 79), 91(27), 79(18), 43(21).



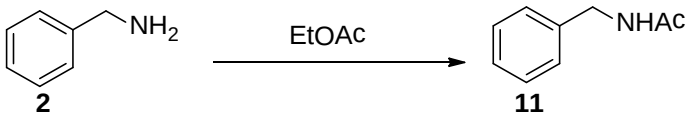
SI-12 : Aza-Michael reactions using CALB on an orbital shaker and microwave irradiation



SI- 13 : Chromatograms obtained by GC-MS. (a) Enzymatic reaction of amine **2** with ketone **8** in hexane under orbital shaking in triplicate (48 h, 33 °C, 130 rpm). (b) Enzymatic reaction of amine **2** with ketone **8** in hexane after extraction into ethyl acetate and water after 24 hours on an orbital shaker (33 °C, 133 rpm). Conditions of GC-MS analysis: DB-5 column (30 m x 0.25 mm x 0.25 µm) Ti = 50 °C, Tf = 270 °C, r = 10 °C/min, t = 32.0 min



SI- 14 : Extraction of aza-Michael reaction with ethyl acetate and water after 24 h on an orbital shaker (33 °C, 133 rpm)



SI-15 : Acetylation of amine **2** by EtOAc