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ARTICLE *in* THE JOURNAL OF ORGANIC CHEMISTRY · FEBRUARY 2005

Impact Factor: 4.72 · DOI: 10.1021/jo048309e · Source: PubMed

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Unexpected Synthesis of Conformationally Restricted Analogues of γ-Amino Butyric Acid (GABA): Mechanism Elucidation by Electrospray Ionization Mass Spectrometry

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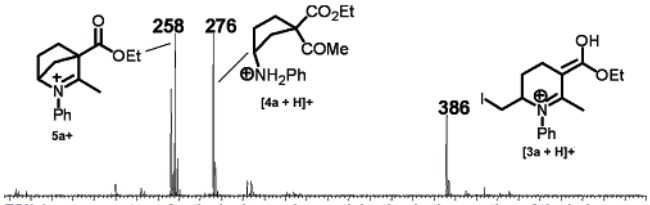
Received September 22, 2004



ESI(+) mass spectrum for the ionic species participating in the reaction of the iodo-β-enamino-ester 3a mediated by Et_3N.

From previous results with lower homologues, dehydroiodination of the three alkenyl-β-enamino esters **3a**−**c** was expected to provide six-membered N-heterocyclic products. The reactions of **3a**−**c** with triethylamine are found to lead, however, to the unexpected stereoselective synthesis of the trisubstituted cyclopentane derivatives **4a**−**c**, as confirmed by IR and NMR spectroscopy. Cyclopentanes **4a**−**c** bear two chiral centers and a γ-amino ester moiety, and are therefore conformationally restricted analogues of γ-amino butyric acid (GABA), which is the major inhibitory neurotransmitter in the central nervous system. Use of electrospray ionization mass (ESI-MS) and tandem mass spectrometry (ESI-MS/MS) allowed the key iminium ion intermediates **5a**−**c**$^+$, as well as the protonated molecules of both the reactant and final products, **[3a**−**c** + **H]**$^+$ and **[4a**−**c** + **H]**$^+$, to be intercepted and structurally characterized. From these findings a mechanism for this unexpected but synthetically attractive and efficient stereoselective reaction is proposed.

Introduction

γ-Amino butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) and is likely present in about 60−70% of all CNS synapses.[1] The synthesis of conformationally restricted GABA analogues is therefore an important current target in organic synthesis, and many attempts have been made to produce GABA model compounds[2] containing chiral centers.

β-Enamino carbonyl compounds are very useful building blocks in the synthesis of many natural products and potential drugs,[3] and their reactivity and synthetic applications have been the subject of continuing studies.[4] We have previously reported[5] the iodine-promoted cyclization of a series of alkenyl-substituted β-enamino esters and ketones, giving the corresponding nitrogen

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(1) (a) Krogsgaard-Larsen, P. *Med. Res. Rev*. **1988**, *8*, 27. (b) Andersen, K. E.; Sorensen, J. L.; Lau, J.; Lundt, B. F.; Petersen, H.; Huusfeldt, P. O.; Suzdak, P. D.; Swedberg, M. D. B. *J. Med. Chem*. **2001**, *44*, 2152.

(2) (a) Moglioni, A. G.; Brousse, B. N.; Álvarez-Larena, A.; Moltrasio, G. Y.; Ortuño, R. M. *Tetrahedron: Asymmetry* **2002**, *13*, 451. (b) Carpes, M. J.; Correia, C. R. D. *Tetrahedron Lett.* **2002**, *43*, 741. (c) Dumond, Y. R.; Montchamp, J. L. *J. Organomet. Chem.* **2002**, *653*, 252. (d) Ito, H.; Omodera, K.; Takigawa, Y.; Taguchi, T. *Org. Lett.* **2002**, *4*, 1499. (e) Aitken, R. A.; Karodia, N.; Massil, T.; Young, R. J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 533. (f) Blay, G.; Fernàndez, I.; Monje, B.; Pedro, J. R. *Tetrahedron* **2004**, *60*, 165.

(3) (a) Eberlin, M. N.; Kascheres, C. *J. Org. Chem.* **1988**, *53*, 2084. (b) Augusti, R.; Eberlin, M. N.; Kascheres, C. *J. Heterocycl. Chem.* **1995**, *32*, 1355. (c) Augusti, R.; Kascheres, C. *J. Org. Chem.* **1993**, *58*, 7079. (d) Fustero, S.; Torre, M. G.; Jofre, V.; Carlón, R. P.; Navarro, A.; Fuentes, A. S.; Carrió, J. S. *J. Org. Chem.* **1998**, *63*, 8825. (e) Gilchrist, T. L. *J. Chem. Soc., Perkin Trans. 1* **1998**, 615.

(4) (a) Ferraz, H. M. C.; Pereira, F. L. C. *Quim. Nova* **2004**, *27*, 89. (b) Kascheres, C. M. *J. Braz. Chem. Soc.* **2003**, *14*, 945. (c) Elassar, A. Z. A.; El-Khair, A. A. *Tetrahedron* **2003**, *59*, 8463. (d) Melo, J. O. F.; Ratton, P. M.; Augusti, R.; Donnici, C. L. *Synthetic Commun.* **2004**, *34*, 369.

(5) Ferraz, H. M. C.; Oliveira, E. O.; Payret-Arrúa, M. E.; Brandt, C. A. *J. Org. Chem.* **1995**, *60*, 7357.

10.1021/jo048309e CCC: $30.25
Published on Web 12/08/2004

SCHEME 1

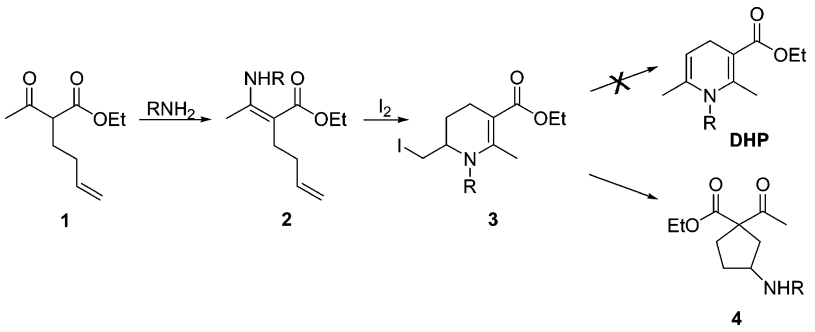
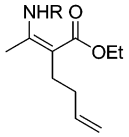
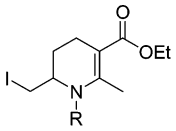


TABLE 1. Reaction Conditions and Yields for the Synthesis of the Cyclic Iodo-β-enamino Esters 3a–c

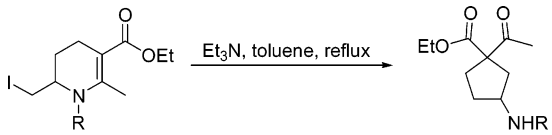
Entry	Substrate	Time (h)	Product	Yield (%)
				
	2a		**3a**	
1	R = Ph	4	R = Ph	78
	2b		**3b**	
2	R = Bn	0.5	R = Bn	60
	2c		**3c**	
3	R = *p*-MeOPh	4	R = *p*-MeOPh	65

five-membered rings. Base-promoted dehydroiodination of these heterocyclic products has been shown to furnish pyrrolic and tetrahydroindolic derivatives.[6]

As a rational extension of these studies, we decided to investigate the iodo-cyclization of homologous alkenyl-β-enamino esters such as **2** (Scheme 1). These homologues are, by analogy, expected to provide six-membered nitrogen heterocyclic products, which should be useful precursors of dihydropyridine moieties (DHP), an important subunit in many compounds with important biological activities.[7] However, as discussed herein, when we treated the cyclic iodo-β-enamino-esters **3** with base, we observed the unexpected but synthetically attractive and effective stereoselective formation of trisubstituted cyclopentane derivatives **4** (Scheme 1). Cyclopentanes **4** are attractive since they bear two chiral centers and a γ-amino ester moiety and can therefore be viewed as conformationally restricted analogues of GABA.

Molecular analysis by mass spectrometry has benefited tremendously from the development of electrospray ionization (ESI),[8] since polar molecules of many types, sizes, forms, and even loosely bonded supramolecules[9] can be easily ionized by ESI for MS investigation. ESI,

SCHEME 2



3a (R = Ph, 72h) **4a** (73%)

3b (R = Bn, 24h) **4b** (85%)

3c (R = p-MeOPh, 72h) **4c** (77%)

an interesting "ion-fishing" technique, gently transfers preformed ions directly from solution to the gas phase, and ESI-MS(/MS) is rapidly becoming the technique of choice for solution mechanistic studies in chemistry and biochemistry[10] and for high-throughput screening of the products from homogeneous catalysis[11] and of metal complexes.[12] We therefore used this technique to monitor the unprecedented reaction leading to the unexpected formation of the GABA analogues **4** in the hope of "fishing" for the key ionic intermediates directly from solution into the gas phase to probe the reaction mechanism.

Results and Discussion

The starting β-enamino ester **2a** was readily synthesized from the known[13] α-alkenylated ethyl acetoacetate **1**, and its iodocyclization to form **3a** was performed by

(6) Ferraz, H. M. C.; Pereira, F. L. C.; Leite, F. S.; Nunes, M. R. S.; Payret-Arrúa, M. E. *Tetrahedron* **1999**, *55*, 10915.

(7) (a) Triggle, D. J.; Langs, D. A.; Janis, R. A. *Med. Res. Rev.* **1989**, *9*, 123. (b) Triggle, D. J.; Janis, R. A. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 347. (c) Elmslie, K. S. *J. Neurosci. Res.* **2004**, *75*, 733. (d) Stout, D. M.; Meyers, A. I. *Chem. Rev*. **1982**, *82*, 233. (e) Sausins, A.; Duburs, G. *Heterocycles* **1988**, *27*, 291. (f) Goldman, S.; Stoltefuss, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1559.

(8) (a) Whitehouse, C. M.; Dreyer, R. N.; Yamashita, M.; Fenn, J. B. *Anal. Chem*. **1985**, *57*, 675. (b) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64. (d) Cole, R. B. *Electrospray Ionization Mass Spectroscopy*; John Wiley & Sons, Inc.: New York, 1997.

(9) (a) Cooks, R. G.; Zhang, D. X.; Koch, K. J.; Gozzo, F. C.; Eberlin, M. N. *Anal. Chem.* **2001**, *73*, 3646. (b) Gozzo, F. C.; Santos, L. S.; Augusti, R.; Consorti, C. S.; Dupont, J.; Eberlin, M. N. *Chem. Eur. J.* **2004**, *10*, 6187.

(10) (a) Koch, K. J.; Gozzo, F. C.; Nanita, S. C.; Takats, Z.; Eberlin, M. N.; Cooks, R. G. *Angew. Chem., Int. Ed.* **2002**, *41*, 1721. (b) Santos, L. S.; Pavam, C. H.; Almeida, W. P.; Coelho, F.; Eberlin, M. N. *Angew. Chem., Int. Ed.* **2004**, *43*, 4330. (c) Raminelli, C.; Prechtl, M. H. G.; Santos, L. S.; Eberlin, M. N.; Comasseto, J. V. *Organometallics* **2004**, *23*, 3990. (d) Domingos, J. B.; Longhinotti, E.; Brandão, T. A. S.; Bunton, C. A.; Santos, L. S.; Eberlin, M. N.; Nome, F. *J. Org. Chem.* **2004**, *69*, 6024. (e) Meyer, S.; Metzger, J. O. *Anal. Bioanal. Chem.* **2003**, *377*, 1108. (f) Meyer, S.; Koch, R.; Metzger, J. O. *Angew. Chem., Int. Ed.* **2003**, *42*, 4700. (g) Meurer, E. C.; Santos, L. S.; Pilli, R. A.; Eberlin, M. N. *Org*. Lett. **2003**, *5*, 1391. (i) Griep-Raming, J.; Meyer, S.; Bruhn, T.; Metzger, J. O. *Angew. Chem.* **2002**, *114*, 2863.

(11) (a) Hilderling, C.; Adlhart, C.; Chen, P. *Angew. Chem., Int. Ed.* **1998**, *37*, 2685. (b) Chen, P. *Angew. Chem., Int. Ed.* **2003**, *42*, 2832.

(12) (a) Tomazela, D. M.; Gozzo, F. C.; Ebeling, G.; Dupont, J.; Eberlin, M. N. *Inorg. Chim. Acta* **2004**, *357*, 2349. (b) Neto, B. A. S.; Ebeling, G.; Gonçalves, R. S.; Gozzo, F. C.; Eberlin, M. N.; Dupont, J. *Synthesis* **2004**, 1155. (c) Pereira, R. M. S.; Paula, V. I.; Buffon, R.; Tomazela, D. M.; Eberlin, M. N. *Inorg. Chim. Acta* **2004**, *357*, 2100.

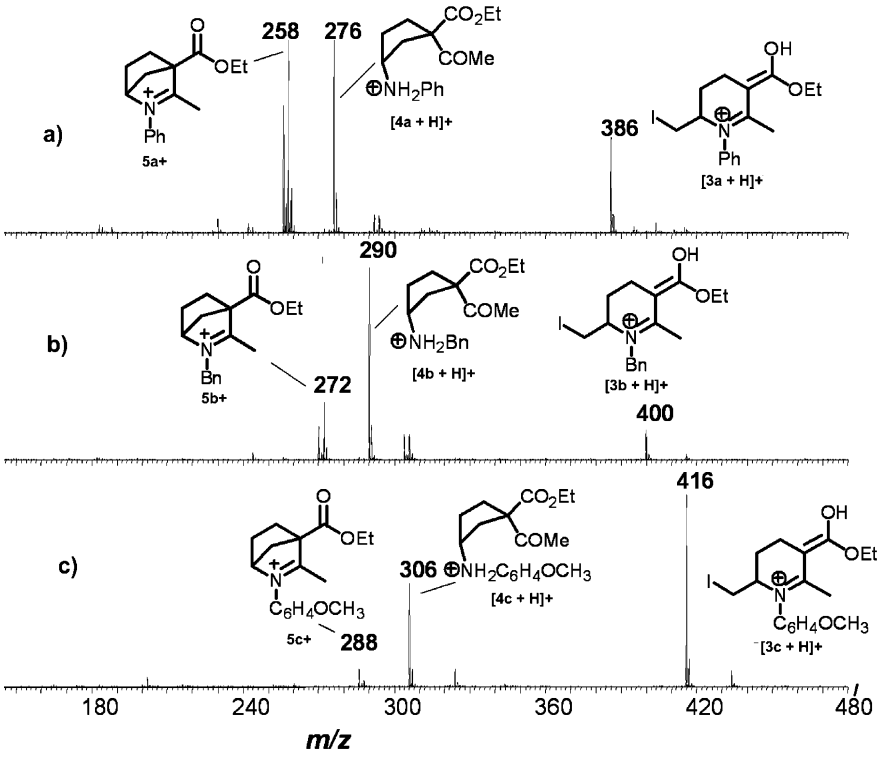
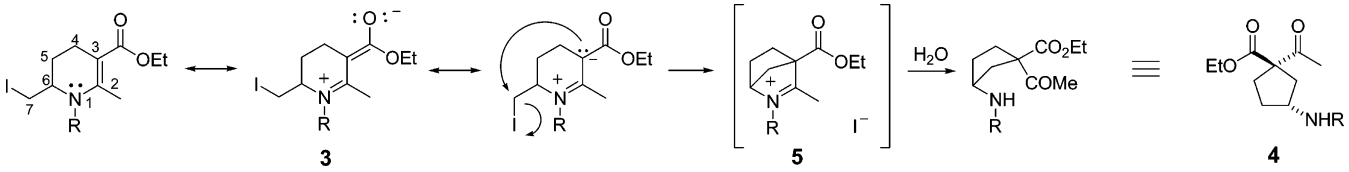


FIGURE 1. ESI(+) mass spectrum for the ionic species participating in the reaction of iodo-β-enamino-esters (a) **3a**, (b) **3b**, and (c) **3c** mediated by Et_3N in 1:1 toluene/methanol solutions containing traces of water. Note the detection of the key bicyclic iminium ion intermediates **5a**−**c**$^+$, as well as both their precursors **[3a**−**c** + **H]**$^+$ and hydrolysis products **[4a**−**c** + **H]**$^+$ in protonated forms (Scheme 3).

SCHEME 3



treatment with iodine, $NaHCO_3$ and CH_2Cl_2 at room temperature (Table 1). To investigate the influence in the course of the iodocyclization of the group attached directly to the nitrogen in **3**, the two β-enamino esters **2b** and **2c** were also prepared and submitted to the same reaction conditions to afford **3b** and **3c** (Table 1).

Treatment of the cyclic compound **3a** with DBU, under the same conditions previously employed[6] for dehydroiodination of the analogous five-membered rings (2 equiv of DBU, toluene, reflux) failed to yield any detectable product, and the starting material was recovered almost quantitatively.

When we changed the base from DBU to Et_3N, however, the reaction furnished a single product in high yield, which was characterized by NMR and IR spectroscopy as the trisubstituted cyclopentane **4a**. We then submitted **3b** and **3c** to the same reaction conditions using Et_3N as the base and likewise obtained the corresponding products **4b** and **4c** in good yields, each as the sole isolable reaction product (Scheme 2).

Scheme 3 rationalizes the transformation of **3** into **4** under basic media. A zwitterionic form of **3** can be viewed to promote intramolecular nucleophilic attack of C_3 onto the iodo-bearing C_7 to give the transient iodide salt **5** of a bicyclic iminium ion (**5**$^+$), which is then rapidly and stereoselectively hydrolyzed yielding the cyclopentane derivative **4**.

We were astonished to find that the unexpected reaction is also stereoseletive, forming a single diastereoisomer of the cyclopentane derivatives **4a**−**c**, as shown by ^{13}C NMR analysis. On the basis of the reaction sequence of Scheme 3, a cis relationship between the amino and the acetyl groups would be the most probable.

Mass Spectrometric Experiments. ESI is known for its ability to transfer ions to the gas phase without inducing undesirable side reactions, and the composition of ESI-generated ions often closely reflects that in solution.[14] Therefore, looking for experimental support to validate the mechanism of ring contraction proposed in Scheme 3, we monitored the reaction by ESI-MS and ESI-MS/MS. A bicyclic iminium ion intermediate **5**$^+$ is proposed to participate in the reaction (Scheme 3), but both reactant **3** and product **4** are neutral molecules. These

(13) Ferraz, H. M. C.; Sano, M. K.; Nunes, M. R. S.; Bianco, G. G. *J. Org. Chem.* **2002**, *67*, 4122.

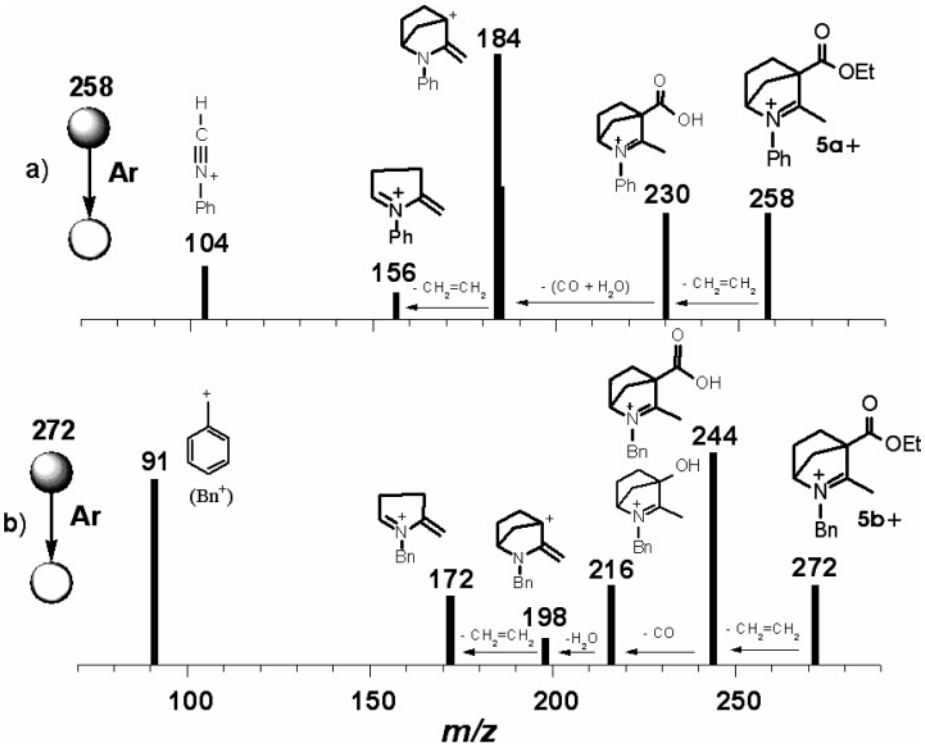
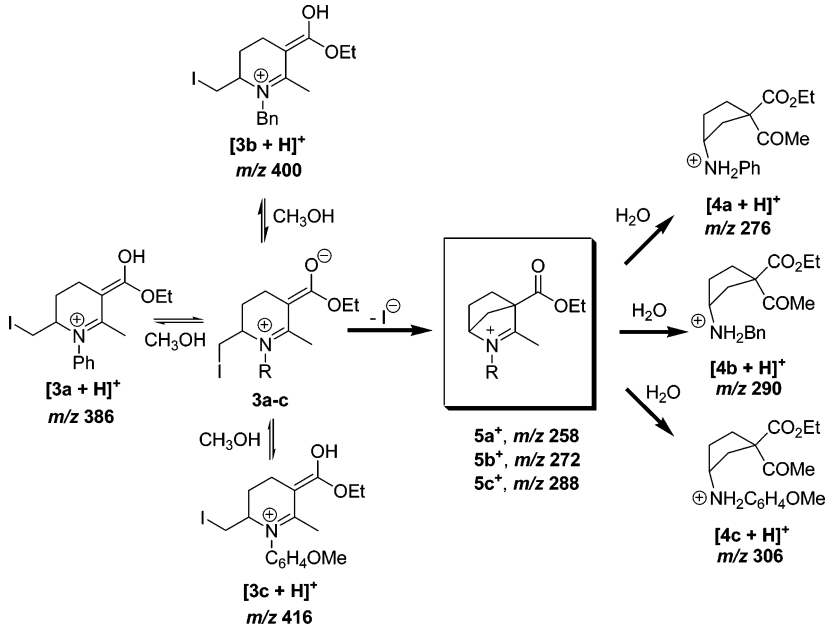


FIGURE 2. ESI(+) tandem mass spectra of the bicyclic iminium ion intermediates (a) **5a**$^+$ of *m/z* 258 and (b) **5b**$^+$ of *m/z* 272. Intermediate **5c**$^+$ of *m/z* 288 has also been detected by ESI-MS (Figure 1c) as a low-abundance peak, but ESI-MS/MS (spectrum not shown) indicates contamination by an isobaric species.

SCHEME 4



neutral species are, however, expected to be in equilibrium in solutions of protic solvents such as methanol with their protonated forms. Therefore, ESI could transfer both reactants and products to the gas phase as $[M + H]^+$ species for MS analysis. Even an unfavored equilibrium would be helpful considering the exceptionally high sensitivity of the ESI-MS technique.[8d]

First we performed the ESI-MS monitoring of the reaction of iodo-β-enamino-esters **3a**−**c** with Et_3N as the base. Iodo-β-enamino-esters **3** (1.0 equiv) and Et_3N (1.0 equiv) were mixed in 1:1 toluene/methanol (2 mL) at 25 °C, and the reaction was monitored by ESI-MS (Figure 1) using a microsyringe pump to deliver the solution directly to the ESI source at flow rates of 0.01 mL min^{-1}. Note that through anhydrous HPLC-grade solvents were used, traces of water, which are needed for the last hydrolysis step that yields **4**, were likely present. Major ions were clearly detected by ESI-MS and correspond, for **3a** (Figure 1a), to the protonated reactant [**3a** + H]$^+$ of *m/z* 386, intermediate **5a**$^+$ of *m/z* 258, and the proto-

nated product [**4a** + H]$^+$ of *m/z* 276. Likewise for **3b** (Figure 1b) and **3c** (Figure 1c), the protonated reactant [**3b** + H]$^+$ of *m/z* 400 and [**3c** + H]$^+$ of *m/z* 416, intermediates **5b**$^+$ of *m/z* 272 and **5c**$^+$ of *m/z* 306, and the final protonated products [**4b** + H]$^+$ of *m/z* 290 and [**4c** + H]$^+$ of *m/z* 306 were clearly detected. The detected cations, as shown by continuous ESI-MS monitoring, were the same from 1 to 60 min of reaction.

For structural characterization, each of these cations, gently transferred to the gas phase and clearly detected by ESI-MS in intact forms, were then mass-selected via collision-induced dissociation (CID) with argon in ESI-MS/MS experiments. Figure 2 shows ESI-MS/MS data for the key bicyclic iminium ion intermediates **5a**$^+$ of *m/z* 258 and **5b**$^+$ of *m/z* 272. These ions dissociate by pathways that match the structures proposed in Scheme 3, as seen by the assignments of fragment ions and neutral losses incorporated into each respective mass spectrum.

Scheme 4 summarizes a general reaction pathway showing neutral and protonated reactants and protonated products, as well as the key bicyclic iminium ion intermediates **5a**−**c**$^+$ (with their respective *m/z* ratios) that have been intercepted and structurally characterized by ESI-MS(/MS).

Conclusion

A novel and straightforward three-step sequence is reported for the efficient and stereoselective synthesis of trisubstituted cyclopentane derivatives **4** from readily accessible α-alkenylated ethyl acetoacetate **1**. A mechanism for this unexpected but synthetically attractive reaction is proposed and further corroborated by ESI-MS(/MS) following the interception and structural characterization of the key bicyclic iminium ion intermediates **5**$^+$. The presence of a γ-amino ester moiety makes the cyclopentanes **4** interesting conformationally restricted analogues of GABA, prompting further investigation of their promising biological activities.

Acknowledgment. We thank the São Paulo State Research Foundation (FAPESP) and the Brazilian National Research Council (CNPq) for financial support.

Supporting Information Available: General experimental methods and spectroscopic data, including NMR spectra of **3a**−**c** and **4a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO048309E