Pergamon

Tetrahedron 56 (2000) 4541–4553

TETRAHEDRON

New Syntheses of *rac*-Huperzine A and its *rac*-7-Ethyl-Derivative. Evaluation of Several Huperzine A Analogues as Acetylcholinesterase Inhibitors

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Received 6 March 2000; revised 3 April 2000; accepted 20 April 2000

Abstract—*rac*-Huperzine A, its *rac*-7-ethyl-derivative and two regioisomeric analogues have been prepared through synthetic sequences involving the elaboration of the pyridone ring in a late stage, by reaction of an intermediate pyrrolidine enamine with propiolamide, which gave mixtures of regioisomeric pyridone derivatives. The acetylcholinesterase inhibitory activity of these and two other recently described 11-unsubstituted huperzine A analogues was determined, the *rac*-7-ethyl analogue of huperzine A being the most active compound, although it is about 12-fold less active than (−)-huperzine A.

Cholinergic neurotransmission is specially affected in patients with Alzheimer's disease.[1–5] Experimental evidence has shown that inhibitors of acetylcholinesterase (AChE) might elevate levels of acetylcholine in the brains of these patients and reverse the cognitive decline.[6–8] The first, and thus far the only, two drugs approved in the USA for the treatment of the cognitive deficit in Alzheimer's disease, i.e. tacrine, **3** (Cognex®) and donepezil (Aricept®), are both reversible inhibitors of AChE. (−)-Huperzine A (**1**), a lycopodium alkaloid isolated from the club moss *Huperzia serrata* (Thunb.) Trev.=*Lycopodium serratum* Thunb. and a Chinese traditional medicine,[9–12] is another potent and selective reversible inhibitor of AChE, which appears to be superior to other AChE inhibitors such as tacrine, physostigmine or galanthamine, because of its comparatively longer duration of action and higher therapeutic index[13] (Fig. 1).

Some time ago, we published the synthesis and pharmacological evaluation of a series of huperzine A-tacrine hybrid compounds.[14] The sole compound of the series which incorporated the ethylidene group characteristic of huperzine A (**4**) was 2.5-fold less potent than tacrine as AChE inhibitor, while compound **5**, lacking the ethylidene substituent, was 2-fold more potent than tacrine, being selected as the lead compound of a more restricted series. Recently we have published the synthesis, pharmacological evaluation, and molecular modeling, of new potent huperzine A-tacrine hybrid compounds resulting from the modification of the parent structure **5**.[15] The more active hybrid compounds of the new series contain a 9-ethyl group and a 3-fluorine atom, or still better a 3-chlorine atom (**6**)[16] (Fig. 1).

When we started this work, we did not have the molecular modeling information and we assumed that our hybrid

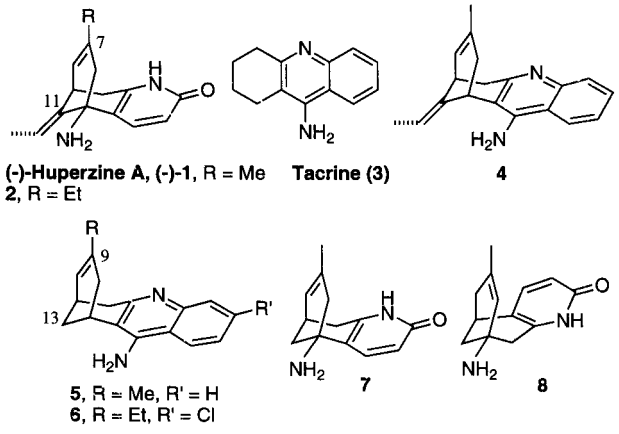

Figure 1. Structures of several acetylcholinesterase inhibitors.

Keywords: biologically active compounds; polycyclic heterocyclic compounds; pyridones; acetylcholinesterase inhibitory activity.
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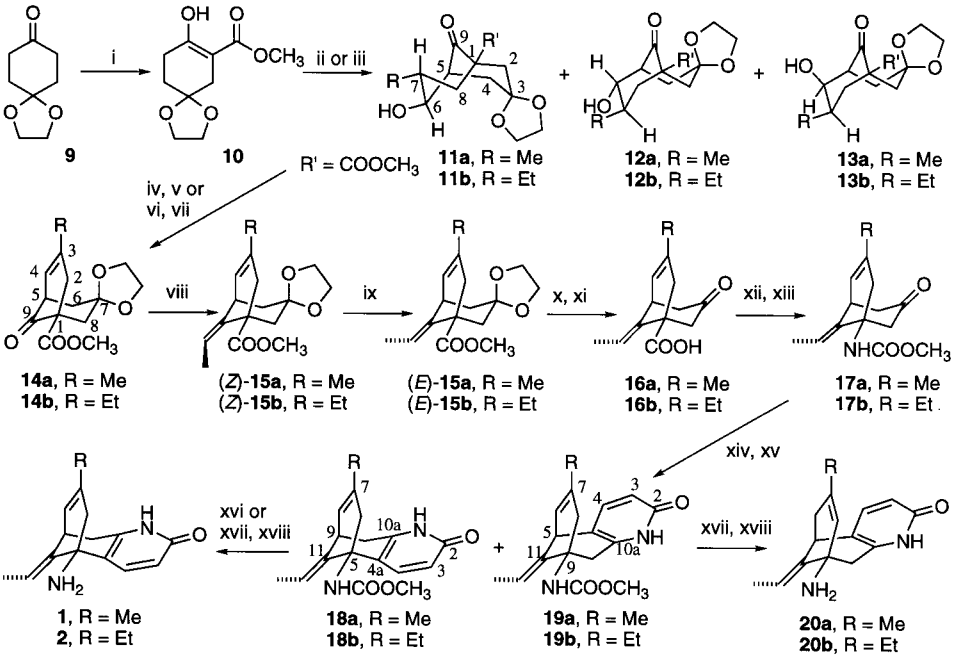
PII: S0040-4020(00)00363-X



i) Me_2CO_3, NaH / KH, THF, reflux, 30 min, 86% yield; ii) α-ethylacrolein, TMG, CH_2Cl_2, -78 °C, 30 min, then -78 °C to room temp. for 3 h and 2 h more at room temp.; iii) α-ethylacrolein, DBU, acetonitrile, room temp., 30 min; iv) *p*-tolyl chlorothionoformate, pyridine, 0 °C to room temp., then room temp., 3 h; v) pyrolysis at 250 °C / 2-4 Torr (**14b** from **10**: 28% yield for ii + iv + v, 27% yield for iii + iv + v); vi) methanesulfonyl chloride, Et_3N, DMAP, CH_2Cl_2, room temp., 6 h; vii) DBU, toluene, reflux, 24 h (**14b** from **13b**: 21% yield for vi + vii); viii) ethyltriphenylphosphonium bromide, *n*-BuLi, THF, room temp., 1.25 h, then **14b** in THF at 0 °C, then room temp., 4 h, 89% yield of (*Z*)-**15b** / (*E*)-**15b** in the ratio of 85:15; ix) thiophenol, AIBN, toluene, 85 °C, 22 h, 86% yield of (*E*)-**15b** / (*Z*)-**15b** in the ratio of 95:5; x) 20% aq. NaOH, THF / MeOH 1:1, reflux, 48 h; xi) 2 N HCl, dioxane, room temp., 4 h, 75% yield of **16b**; xii) $(C_6H_5O)_2P(O)N_3$, Et_3N, chlorobenzene, 90 °C, 3.5 h; xiii) MeOH, reflux, 17 h (**17b** from **16b**: 83% yield for xii + xiii); xiv) pyrrolidine, 4 Å molecular sieves, benzene, reflux, 5 h; xv) propiolamide, reflux, 15-21 h (40% yield of **18a** and 26% yield of **19a** for xiv + xv from **17a**, taking into account the recovered starting keto carbamate; 28% yield of **18b** and 19% yield of **19b** for xiv + xv from **17b**, taking into account the recovered starting keto carbamate); xvi) *n*-PrSLi, HMPA, 40 °C, 24 h, 66% yield of **1**; xvii) TMSI, $CHCl_3$, reflux, 8 h; xviii) MeOH, reflux, 14 h (61% yield of **2**, quantitative yield of **20a** and 85% yield of **20b** for xvii + xviii).

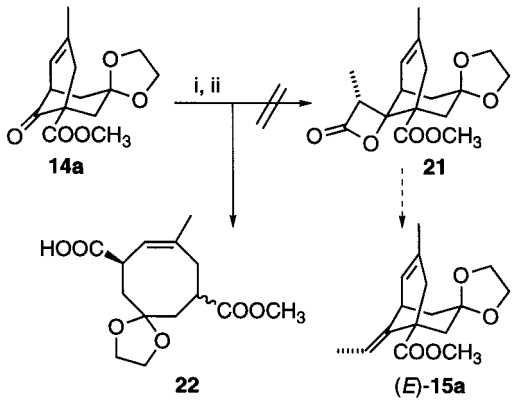
Scheme 1. Synthesis of *rac*-huperzine A, **1**, its *rac*-7-ethyl-derivative, **2**, and their regioisomers **20a** and **20b**.

compounds were placed in the active site of AChE in a similar environment to that of huperzine A in such a way that their carbobicyclic substructures would occupy roughly the same position and that the 4-aminoquinoline substructure of the hybrid compounds would partly occupy the same position of the 2-pyridone subunit of huperzine A. Taking into account the pharmacological data of the hybrid compounds, we planned the synthesis of two huperzine A analogues, which could be more active than huperzine A: one of them lacking the ethylidene substituent at position 11 (**7**) (Fig. 1) and the other one having a 7-ethyl instead of a 7-methyl group (**2**). Although compound **2** was protected in a patent[17] and it has been recently cited,[18] to the best of our knowledge, its synthesis and activity data have not been published yet.

Our initial work on the synthesis of new analogues of huperzine A had been directed to the preparation of analogues modified at the heterocyclic moiety. At that moment, all the described syntheses of huperzine A and its analogues started from compounds containing a protected 2-pyridone ring. We envisioned a route to a common advanced intermediate, such as keto carbamate **17a** (Scheme 1), from which we could gain easy access to different modified heterocyclic analogues of huperzine A by elaboration of the heterocyclic ring from the ketone functionality of **17a** followed by deprotection of the bridgehead amino group.

When we had prepared keto acid **16a**, precursor of **17a**, Kozikowski et al. published the synthesis of a 2-aminothiazole analogue of huperzine A from keto carbamate **17a**.[19] They had prepared the last compound through a synthetic sequence parallel to the one we were developing. This fact prompted us to publish our improved conversion of keto ester **10** into the bicyclic compound **14a**,[20] the only part of our synthetic sequence that was somewhat different from the published one. Worthy of note, the attempted conversion of **17a** to the 2-aminothiazole analogue of huperzine A by Kozikowski's group did not give the desired product but a regioisomeric derivative, in which ring closure had taken place from the less hindered α-carbonyl position.[19]

In spite of the above discouraging results, we completed the conversion of **17a** to *rac*-huperzine A and applied the new methodology to the synthesis of the *rac*-7-ethyl-derivative of huperzine A, **2**, which is herein described. The synthesis of the 11-deethylidene analogue **7** and its regioisomer **8** (Fig. 1) using a similar methodology, has been recently



Scheme 2. Attempted stereoselective synthesis of (*E*)-**15a** from **14a** via **21**. i) *S*-phenyl thiopropionate, LDA, THF, −78°C, 30 min; ii) **14a**, THF −78°C, 30 min, then 1.5 h from −78 to 0°C; 69% yield of **22** as a 55:45 diastereomeric mixture.

published.[21] Also, we report herein the AChE inhibitory activity of all of the different huperzine A analogues prepared in our group.

Results and Discussion

Keto carbamate **17a** was obtained in 22% overall yield from commercially available 1,4-cyclohexanedione monoethylene ketal, **9** (Scheme 1) as described by Kozikowski et al.,[19] except for the conversion of the stereoisomeric mixture of alcohols **11a** and **12a** to compound **14a**, which was carried out as previously described by our group.[20]

Although compound (*E*)-**15a** had been obtained in high yield and high diastereomeric purity from **14a** by Wittig olefination to a 85:15 mixture of (*Z*)-**15a** and (*E*)-**15a**, followed by isomerization of the exocyclic C=C double bond, alternatively we attempted the stereoselective conversion of **14a** to (*E*)-**15a**, through a two-step protocol that had been successfully used for this purpose in the synthesis of other huperzine A analogues. This involved the diastereoselective formation of an intermediate β-lactone, followed by silica gel-promoted stereospecific [2+2]-retrocycloaddition of this β-lactone with evolution of CO_2.[22] However, treatment of **14a** with the enolate derived from *S*-phenyl thiopropionate did not lead to the desired β-lactone, but to a product of retro-Dieckmann reaction, **22**, as a diastereomeric mixture, which was isolated in 69% yield after column chromatography (Scheme 2).

Elaboration of the pyridone ring from keto carbamate **17a** was carried out by using a modification of a procedure described by Kozikowski et al.[23] Reaction of **17a** with pyrrolidine in refluxing benzene in the presence of 4 Å molecular sieves, gave the corresponding enamine, for which Kozikowski's group had established an *anti*-arrangement for the endocyclic C=C double bonds.[19] Reaction of this enamine with freshly prepared propiolamide (from ethyl propiolate and concentrated ammonium hydroxide)[24] in benzene under reflux afforded a mixture of regioisomeric pyridones **18a** and **19a**, in which the first one was the major regioisomer. These pyridones were efficiently separated by column chromatography through silica gel, a significant amount of starting **17a** (32%) being recovered (40% yield of **18a** and 26% yield of **19a**, taking into account the recovered starting keto carbamate). Pyridone **18a** is a known product which had been obtained in low yield by Kozikowski's group as a by-product in the first synthesis of huperzine A,[25,26] while **19a** is a new product, which was fully characterized through its spectroscopic data and elemental analysis.

Cleavage of the methyl carbamate function of **18a** was carried out by reaction with lithium 1-propanethiolate in hexamethylphosphoric triamide (HMPA),[27] obtaining huperzine A, **1**, in 66% yield, after twofold column chromatography. According to our experience in a related case,[21] the cleavage of the carbamate group of **19a** was not attempted under the above conditions and was carried out directly by reaction with trimethylsilyl iodide (TMSI) followed by reaction with MeOH under reflux,[28] obtaining in quantitative yield the new huperzine A analogue **20a**, resulting from the hydrolysis of the carbamate function and the HI-promoted isomerization of the endocyclic

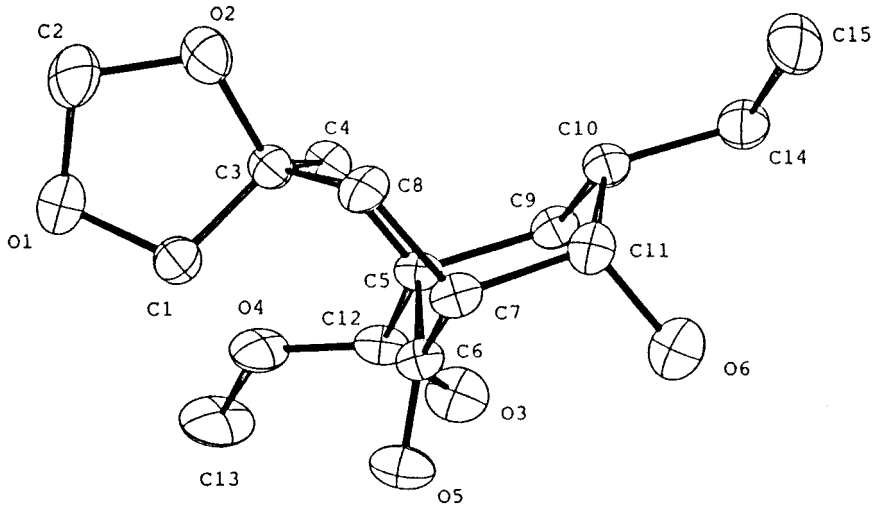


Figure 2. Crystal structure (ORTEP) of alcohol **13b**.

Table 1. AChE inhibitory activity of (−)-huperzine A and its analogues **2**, **7**, **8**, **20a**, and **20b**. Values are expressed as mean±standard error of the mean of at least four experiments. IC_{50}, 50% inhibitory concentration of AChE (from bovine erithrocytes) activity

Compound	IC_{50} (μM)
Tacrine, **3**	0.130±0.010
(−)-Huperzine A, (−)-**1**	0.074±0.0055
2	0.870±0.081
7	9.05±0.40
8	>100
20a	25.1±0.4
20b	97.1±5.8

C═C double bond to a neighboring position, what reflects the greater stability of the *anti*-arrangement for the endocyclic C═C double bond and the heterocyclic ring in this kind of bicyclo[3.3.1]nonadiene derivatives.[19]

For the synthesis of the *rac*-7-ethyl analogue of huperzine A, through the above methodology, compound **10** was reacted with α-ethylacrolein in the presence of *N,N,N′,N′*-tetramethylguanidine (TMG) as the base catalyst. The product thus obtained (probably a mixture of alcohols **11b** and **12b** according to our previous results in the corresponding reaction with methacrolein[20]) was used as such in the next step. Reaction with *p*-tolyl chlorothionoformate, followed by pyrolysis of the resulting mixture of thiocarbonates at 250°C/2–4 Torr led to the olefin **14b** in 28% overall yield. When the reaction of **10** with α-ethylacrolein was carried out in the presence of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) as the base catalyst, a crude product containing mainly alcohols **12b** and **13b** was obtained (^{13}C NMR). Column chromatography of this crude product gave in order of elution an impure mixture of **12b** and **13b** in an approximate ratio of 2:1 and pure alcohol **13b**.

The relative configuration and the solid state conformation of **13b** were determined by X-ray diffraction analysis (Fig. 2). The hydroxy- and ethyl-bearing cyclohexane ring in **13b** adopts a *chair* conformation, in which these substituents are in a relative *cis*-arrangement with an *axial*-hydroxy group and an *equatorial* ethyl group, while the other cyclohexane ring adopts a *boat* conformation to avoid the steric interaction between the 3-*endo* and 7-*endo* substituents. Worthy of note, a similar compound (**13a**) had not been previously observed in the corresponding reaction with methacrolein, in which a mixture of **11a** and **12a** was mainly obtained when TMG was used as the base catalyst, while mainly **12a** was obtained by using DBU. Since in both **11a** and **12a**, the hydroxy and methyl groups are in a *trans*-arrangement, a pyrolytic *syn* elimination of the corresponding thiocarbonates was used to carry out the dehydration of these alcohols to **14a**, instead of using an elimination reaction via the corresponding mesylates.

Taking advantage of the *trans-diaxial* relationship of the hydroxy group and the proton at position 7 in alcohol **13b**, this compound was transformed into the olefin **14b** by base-induced (DBU) elimination of the corresponding mesylate, although the global yield of **14b** from **13b** was very low (21%). On the other hand, conversion of the mixture of **12b** and **13b** into the corresponding mixture of thiocarbonates, followed by pyrolysis gave **14b** in 27% overall yield from **10**. Wittig reaction of **14b** with ethylidenetriphenylphosphorane afforded a mixture of (*Z*)-**15b** and (*E*)-**15b** in the approximate ratio of 85:15, in 89% yield. Different attempts to obtain pure (*Z*)-**15b** by column chromatography or by distillation were fruitless. However, we could obtain pure (*Z*)-**15b** by preferential saponification of (*E*)-**15b** from the above mixture by using excess of aqueous NaOH in a 1:1 mixture of tetrahydrofuran (THF) and methanol (MeOH).

Isomerization of the mixture of (*Z*)-**15b** and (*E*)-**15b** in the approximate ratio of 85:15 by reaction with thiophenol catalyzed by azobisisobutyronitrile (AIBN) afforded in good yield a mixture of (*E*)-**15b** and (*Z*)-**15b** in the approximate ratio of 95:5, from which pure (*E*)-**15b** could not be obtained by distillation.

Saponification of the mixture of (*E*)-**15b** and (*Z*)-**15b** in the ratio of 95:5 by reaction with 20% aqueous NaOH in a mixture of THF/MeOH in the ratio of 1:1 under reflux,[28] followed by hydrolysis of the ketal function with 2 N HCl in dioxane, afforded pure keto acid **16b** in 75% yield, after column chromatography. A modified Curtius rearrangement of this keto acid **16b** by reaction with diphenylphosphoryl azide in the presence of Et_3N, followed by methanolysis of the resulting isocyanate,[27] afforded keto carbamate **17b** in 83% overall yield.

Elaboration of the 2-pyridone ring from **17b** was carried out in a similar way to that described before for the preparation of pyridones **18a** and **19a**. After a tedious chromatographic purification of the resulting crude product, the expected regioisomeric pure pyridones **18b** and **19b** were isolated in 28% and 19% yield, respectively, taking into account the amount of recovered keto carbamate **17b** (19%).

The higher yield observed for the cleavage of the carbamate group of **19a** with TMSI in comparison with that obtained in the cleavage of **18a** with lithium 1-propanethiolate led us to carry out the cleavage of the methyl carbamate of pyridones **18b** and **19b** under the former reaction conditions. Thus, reaction of **18b** and **19b** with TMSI in refluxing chloroform for 8 h, followed by treatment with methanol under reflux for 14 h, afforded crude products containing the expected amines **2** and **20b**, respectively. Again, the purification of the reaction products proved to be a difficult task, as a consequence of their high polarity, due to the presence of the pyridone ring and the amino group. After successive purifications by column chromatography, the 7-ethyl analogues of huperzine A **2** and **20b** were obtained in 61% and 85% yield, respectively.

All of the new huperzine A analogues prepared in this work, i.e. the regioisomeric huperzine A **20a** and the 7-ethyl analogues **2** and **20b**, as well as the 11-unsubstituted analogues **7** and **8**, described in a previous work,[21] were tested for their ability to inhibit AChE from bovine erythrocytes by the method of Ellman et al.[29] The IC_{50} values for AChE inhibition by these compounds are displayed in Table 1, together with the IC_{50} of tacrine and natural (−)-huperzine A. The sole huperzine A analogue with a significant AChE inhibitory activity is the 7-ethyl analogue **2**, which is 12-fold less potent than (−)-huperzine A. Taking into account the racemic nature of **2** and the fact that *rac*-huperzine A is

Table 2. ^{1}H NMR chemical shifts (all of these spectra were taken at 500 MHz in $CDCl_3$) and coupling constants of compounds **14b**, (*Z*)-**15b**, (*E*)-**15b**, **16b**, and **17b**

	14b	(*Z*)-**15b**	(*E*)-**15b**	**16b**	**17b**
2-H_{exo}	3.30	2.94	2.92	3.00	2.32
2-H_{endo}	2.68	2.16	2.16	2.12	2.32
4-H	5.40	5.40	5.38	5.39	5.35
5-H	2.87	2.79	3.33	3.60	3.58
6-H_{exo}	2.27	1.91	1.81	2.50	2.48
6-H_{endo}	2.13	1.82	1.86	2.45	2.39
8-H_{exo}	2.79	2.39	2.36	3.11	3.43
8-H_{endo}	2.20	1.88	1.91	2.42	2.48
1′-H[a]	2.06	1.95	1.95	1.92	1.88
2′-H[a]	1.07	0.99	0.99	0.95	0.93
1″-H[b]		5.41	4.93	5.41	5.43
2″-H[b]		1.42	1.61	1.75	1.73
OCH_2CH_2O	3.89	3.84	3.83		
	4.02				
OCH_3	3.82	3.72	3.73		3.65
COOH				9.00–13.4	
NHCOO					4.73
J (Hz)					
2-H_{exo}/2-H_{endo}	17.5	17.5	17.5	18.0	
2-H_{exo}/8-H_{exo}	1.0	1.0	1.5	2.0	
4-H/5-H	6.0	6.0	6.0	5.5	5.5
5-H/6-H_{exo}	5.5	5.0	4.5	4.5	4.5
5-H/6-H_{endo}	2.5	2.5	2.5	2.5	2.5
6-H_{exo}/6-H_{endo}	14.0	13.0	13.0	14.5	14.5
6-H_{endo}/8-H_{endo}	3.0	2.5	2.5	2.0	2.5
8-H_{exo}/8-H_{endo}	14.5	14.0	14.0	15.5	13.5
1′-H/2′-H[a]	7.5	7.5	7.5	7.5	7.5
1″-H/2″-H[b]		7.5	7.0	6.5	7.0

[a] 1′-H and 2′-H correspond to the α and β protons of the C3-substituent.
[b] 1″-H and 2″-H correspond to the α and β protons of the C9-substituent.

about one-half as potent as (−)-huperzine A, the acetylcholinesterase inhibitory activity of **2** is only about 6-fold lower than that of *rac*-huperzine A. The 11-unsubstituted huperzine A analogue **7** is 122-fold less potent than (−)-huperzine A, while the regioisomeric analogues **8**, **20a**, and **20b** are still less potent, about 300–1300-fold less potent than (−)-huperzine A.

Table 3. ^{13}C NMR chemical shifts (all of these spectra were taken at 75.4 MHz in $CDCl_3$) of compounds **14b**, (*Z*)-**15b**, (*E*)-**15b**, **16b**, and **17b**

	14b	(*Z*)-**15b**	(*E*)-**15b**	**16b**	**17b**
C1	56.1	47.0	50.6	52.2	57.9
C2	42.0	40.1	39.7	40.7	46.1
C3	140.3	138.5	139.2	138.0	137.6
C4	120.0	122.2	120.9	121.9	122.9
C5	44.4	43.4	32.0	33.2	32.9
C6	41.1	41.0	40.2	46.9	46.2
C7	106.4	108.3	108.5	209.1	208.8
C8	44.5	45.1	45.3	51.6	53.2
C9	209.8	137.8	139.2	136.3	136.4
C1′[a]	29.0	29.3	29.4	29.2	28.9
C2′[a]	12.0	12.0	12.0	12.1	12.1
C1″[b]		115.2	113.3	116.7	113.9
C2″[b]		12.4	13.1	13.2	12.9
COO	171.8	176.7	175.6	179.7	155.1
OCH_3	52.6	52.1	52.0		51.9
OCH_2CH_2O	63.3	62.9	62.9		
	64.6	64.5	62.9		

[a] C1′ and C2′ correspond to the α and β carbon atoms of the C3-substituent.
[b] C1″ and C2″ correspond to the α and β carbon atoms of the C9-substituent.

In conclusion, structural changes on the carbobicyclic substructure that lead to hybrid compound **6** from **4** with improved AChE inhibitory activity (i.e. removal of the ethylidene group at position 11 and substitution of the 9-methyl by a 9-ethyl group), when carried out on the corresponding subunit of huperzine A do not have the same effect. Thus, the removal of the ethylidene group of huperzine A leads to an important decrease of the AChE inhibitory activity, showing clearly that this exocyclic olefinic appendage is one of the essential features for high AChE inhibitory activity,[26,30–34] while the substitution of the 7-methyl group of huperzine A by a 7-ethyl group has a small but negative effect on the AChE inhibitory activity.

The recently published results[15,35] on the molecular modeling of hybrid compounds such as **5** with AChE from *Torpedo californica* (*Tc*AChE) have shown them to interact with the active site of the enzyme as truly huperzine A-tacrine hybrids, but in a different way from that we had assumed. Thus, the 4-aminoquinoline subunit of the more active enantiomer (eutomer) of these compounds [(−)-enantiomer] is placed in the active site of the enzyme in the same position of the corresponding subunit in tacrine, while their unsaturated methyl-substituted three-carbon bridge roughly occupies the same position of the corresponding moiety of natural (−)-huperzine A, in agreement with their absolute configurations,[36] which are opposed, in spite of being all of them levorotatory. The 2-pyridone subunit of (−)-huperzine A occupies a position opposed to that occupied by the 4-aminoquinoline substructure of the hybrid compounds and similarly the ethylidene-substituted

Table 4. ^{1}H NMR chemical shifts (except where otherwise stated, these spectra were taken at 500 MHz in CD_3OD; the values indicated with * within a column can be interchanged) and coupling constants of compounds **1**, **2**, **18a**, and **18b**

	1[a]	**2**	**18a**[b]	**18b**
1-H	12.38	4.87	4.84	4.84
3-H	6.39	6.38	6.34	6.34
4-H	7.88	7.88	7.54	7.54
6-H_{exo}	2.14*	2.31	2.39	2.40
6-H_{endo}	2.09*	2.21	2.13	2.16
8-H	5.39	5.46	5.46	5.46
9-H	3.58	3.70	3.64	3.66
10-H_{exo}	2.87	2.82	2.87	2.89
10-H_{endo}	2.71	2.61	2.57	2.58
1′-H[c]	1.52	1.87	1.55	1.85
2′-H[c]		0.88		0.86
1″-H[d]	5.46	5.54	5.31	5.32
2″-H[d]	1.65	1.72	1.66	1.67
5-NH/5-NH_2	1.58	4.87	4.84	4.84
OCH_3			3.57	3.57
J (Hz)				
3-H/4-H	9.5	9.2	9.0	9.5
6-H_{exo}/6-H_{endo}	17.0	16.5	16.0	16.0
8-H/9-H	5.5	4.0	5.0	4.0
9-H/10-H_{exo}	5.2	5.0	5.0	5.0
9-H/10-H_{endo}	1.5	1.5	2.0	1.5
10-H_{exo}/10-H_{endo}	17.0	17.0	17.0	17.0
1′-H/2′-H[c]		7.5		7.5
1″-H/2″-H[d]	6.7	6.5	7.0	7.0

[a] This spectrum was recorded in $CDCl_3$.
[b] The following coupling constant was also observed: 8-H/1′-H=0.5 Hz.
[c] 1′-H and 2′-H correspond to the α and β protons of the C7-substituent.
[d] 1″-H and 2″-H correspond to the α and β protons of the C11-substituent.

Table 5. ^{13}C NMR chemical shifts (except where otherwise stated, these spectra were taken at 75.4 MHz in CD_3OD; the values indicated with * within a column can be interchanged) of compounds **1**, **2**, **18a**, and **18b**

	1[a]	**2**	**18a**	**18b**
C2	165.4	165.7	165.9	165.8
C3	117.0	117.9	117.9	117.9
C4	140.2	141.4	141.1	141.1
C4a	122.8	124.3	122.7	122.6
C5	54.3	55.4	58.3	58.4
C6	49.1	47.9	48.6	47.0
C7	134.1	140.8	134.1	139.8
C8	124.2	123.6	125.9	124.3
C9	32.8	33.9	34.1	34.0
C10	35.2	36.4	35.1	35.2
C10a	143.1	144.5	144.3	144.1
C11	142.5	141.5	136.6	136.8
C1′[b]	22.6	30.3	22.6	30.2
C2′[b]		12.6*		12.6
C1″[c]	111.2	113.1	113.4	113.4
C2″[c]	12.3	12.7*	12.5	12.5
COO			157.2	157.2
OCH_3			52.3	52.3

[a] This spectrum was recorded in $CDCl_3$.
[b] C1′ and C2′ correspond to the α and β carbon atoms of the C7-substituent.
[c] C1″ and C2″ correspond to the α and β carbon atoms of the C11-substituent.

Table 6. ^1H NMR chemical shifts (all of these spectra were taken at 500 MHz in CD_3OD; the values indicated with * within a column can be interchanged) and coupling constants of compounds **19a**, **b** and **20a**, **b**

	19a	**19b**	**20a**	**20b**
1-H	4.85	4.85	4.84	4.87
3-H	6.29	6.29	6.37	6.39
4-H	7.42	7.43	7.48	7.50
5-H	4.11	4.14	3.99	4.05
6-H	5.58	5.57		
6-H_{exo}			2.43	2.49
6-H_{endo}			2.02	2.11
8-H			5.30	5.34
8-H_{exo}	2.42	2.42		
8-H_{endo}	2.37	2.37		
10-H_{exo}	2.88	2.84	2.59*	2.74*
10-H_{endo}	3.49	3.49	2.74*	2.84*
1′-H[a]	1.63	1.95	1.57	1.91
2′-H[a]		0.96		0.91
1″-H[b]	5.22	5.20	5.52	5.47
2″-H[b]	1.68	1.67	1.72	1.76
9-NH/9-NH_2	4.85	4.85	4.84	4.87
OCH_3	3.62	3.61		
J (Hz)				
3-H/4-H	9.5	9.0	9.2	9.2
5-H/6-H	5.5	6.0		
5-H/6-H_{exo}			4.7	5.0
5-H/6-H_{endo}			1.7	1.5
6-H_{exo}/6-H_{endo}			17.0	17.5
8-H_{exo}/8-H_{endo}	16.0	16.0		
8-H_{exo}/10-H_{exo}				
10-H_{exo}/10-H_{endo}	18.5	18.0	17.0	16.5
1′-H/2′-H[a]		7.5		7.5
1″-H/2″-H[b]	6.5	7.0	6.7	7.0

[a] 1′-H and 2′-H correspond to the α and β protons of the C7-substituent.
[b] 1″-H and 2″-H correspond to the α and β protons of the C11-substituent.

methane-bridge of (−)-huperzine A occupies a position opposed to that occupied by the corresponding bridge of the hybrid compounds. Thus, no structure-activity parallelism should be expected for modifications carried out at C11 of huperzine A and at C13 of the hybrid compounds. However, a certain parallelism might be expected for substitutions at C7 of huperzine A and at C9 of the hybrid compounds, as really observed.

Experimental

General

Melting points were determined with a MFB 595010 M Gallenkamp melting point apparatus. 500 MHz ^1H NMR spectra were performed on a Varian VXR 500 spectrometer, and 75.4 MHz ^{13}C NMR spectra on a Varian Gemini 300. Chemical shifts (δ) are reported in ppm related to internal tetramethylsilane (TMS). Assignments given for the NMR spectra are based on DEPT, $^1H/^1H$ and $^1H/^{13}C$ COSY experiments (HMQC sequence). The ^1H and ^{13}C NMR data of compounds **14b**, (*Z*)-**15b**, (*E*)-**15b**, **16b**, and **17b** are collected in Tables 2 and 3, respectively, the ^1H and ^{13}C NMR data of compounds **1**, **2**, **18a**, and **18b** are collected in Tables 4 and 5, the ^1H and ^{13}C NMR data of compounds **19a**, **19b**, **20a**, and **20b** are collected in Tables 6 and 7, respectively, while those of compounds **12b**, **13b**, and **22** are described in the experimental. IR spectra were recorded on a FT/IR Perkin–Elmer spectrometer, model 1600. Routine MS spectra were taken on a Hewlett–Packard 5988A spectrometer using the electron impact technique (70 eV): only significant ions are given. Unless otherwise stated, silica gel SDS 60 (60–200 μm) was utilized for the column chromatography. Elemental analyses and high resolution mass spectra were carried out, respectively, at the Microanalysis Service and the Mass Spectrometry Laboratory of the Centro de Investigación y Desarrollo (C.I.D.), C.S.I.C., Barcelona, Spain. All new compounds

Table 7. ^{13}C NMR chemical shifts (all of these spectra were taken at 75.4 MHz in CD_3OD) of compounds **19a**, **b** and **20a**, **b**

	19a	**19b**	**20a**	**20b**
C2	165.5	165.5	165.6	165.5
C3	116.9	116.9	118.1	118.4
C4	142.7	142.6	144.4	144.3
C4a	122.2	122.2	120.7	120.4
C5	38.5	38.4	36.4	36.3
C6	126.5	124.8	40.8	39.1
C7	132.7	138.2	135.1	141.7
C8	49.9	48.3	130.6	127.4
C9	56.5	56.6	54.1	54.7
C10	41.9	41.9	44.2	43.6
C10a	145.3	145.4	143.4	142.8
C11	137.0	137.3	141.0	139.9
C1′[a]	22.6	30.2	22.8	30.3
C2′a[b]		12.6		12.4
C1″[b]	112.5	112.3	113.1	113.5
C2″[b]	13.0	12.9	12.6	12.6
COO	157.8	157.8		
OCH_3	52.3	52.2		

[a] C1′ and C2′ correspond to the α and β carbon atoms of the C7-substituent.
[b] C1″ and C2″ correspond to the α and β carbon atoms of the C11-substituent.

herein described are racemic, although this is not indicated either in their names or in their numbering.

(*E*)-5-Amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methano-1*H*-cycloocta[*b*]pyridin-2-one, *rac*-huperzine A, (1). A mixture of carbamate **18a** (150 mg, 0.50 mmol), anhydrous HMPA (0.9 mL) and freshly prepared lithium 1-propanethiolate (ca. 0.5 M solution in anhydrous HMPA,[37] 6.7 mL, ca. 3.35 mmol) was stirred at 40°C for 24 h. The mixture was allowed to cool to room temperature, was poured into crushed ice (50 g) and was concentrated to dryness in vacuo. The resulting brown residue (660 mg) was submitted to column chromatography (40–60 μm-silica gel, 220 g, mixtures of $CHCl_3$/MeOH). On elution with $CHCl_3$/MeOH in the ratio of 95:5, slightly impure **1** (105 mg) was obtained. This product was submitted again to column chromatography (40–60 μm-silica gel, 10 g) under the same conditions, obtaining pure **1** (80 mg, 66% yield), as a white solid, mp 217–219°C (AcOEt). IR (KBr) ν 3358, 3293, 2917, 2894, 1651, 1617, 1554, 1459, 1435, 1405, 1378, 1351, 1309, 1170, 1119, 1087, 959, 926, 910, 841, 771, 720, 659 cm^{-1}.

(*E*)-5-Amino-7-ethyl-11-ethylidene-5,6,9,10-tetrahydro-5,9-methano-1*H*-cycloocta[*b*]pyridin-2-one (2). To a suspension of carbamate **18b** (118 mg, 0.38 mmol) in $CHCl_3$ (14 mL), TMSI (0.54 mL, 0.76 g, 3.8 mmol) was added dropwise and the mixture was heated under reflux for 8 h. MeOH (14 mL) was then added and the reaction mixture was heated under reflux for 14 h. Evaporation of the solvents at reduced pressure gave a brown residue (230 mg), which was submitted to column chromatography (40–60 μm-silica gel, 20 g, mixtures of $CHCl_3$/MeOH). On elution with a mixture of $CHCl_3$/MeOH in the ratio of 96:4, impure amine **2** (130 mg) was obtained as a yellowish solid. This product was again submitted to column chromatography (40–60 μm-silica gel, 20 g, mixtures of hexane/AcOEt and then AcOEt/MeOH). On elution with a mixture of AcOEt/MeOH in the ratio of 91:9, almost pure **2** (59 mg, 61% yield) was obtained as a white solid. The analytical sample of **2** was obtained by recrystallization from MeOH, mp 102–104°C. IR (KBr) ν 3430, 3271, 3142, 2959, 2925, 2863, 1657, 1629, 1609, 1552, 1449, 1427, 1408, 1382, 1350, 1303, 1176, 1120, 972, 929, 902, 835, 773, 717, 662 cm^{-1}. MS, *m/z* (%): 257 [$(M+H)^+$, 22], 256 ($M^{\cdot+}$, 72), 255 [$(M-H)^+$, 12], 242 (15), 241 [$(M-CH_3)^+$, 83], 228 (19), 227 [$(M-CH_2CH_3)^+$, 100], 187 [$(M-C_5H_9)^+$, 81], 173 [$(M-C_6H_{11})^+$, 31], 55 (36). Exact mass calcd for $C_{16}H_{20}N_2O$ 256.1576, obsd 256.1567.

Methyl 7*exo*-ethyl-3,3-ethylenedioxy-6*exo*-hydroxy-9-oxobicyclo[3.3.1]nonane-1-carboxylate (13b) and stereoisomeric mixture of methyl 7*exo*-ethyl-3,3-ethylenedioxy-6*endo*-hydroxy-9-oxobicyclo[3.3.1]nonane-1-carboxylate (12b) and 13b. A solution of **10** (24.0 g, 112 mmol) and DBU (19.6 mL, 20.0 g, 131 mmol) in anhydrous acetonitrile (450 mL) was treated dropwise with a solution of α-ethylacrolein (43.1 mL, 37.0 g, 441 mmol) in anhydrous acetonitrile (200 mL). The reaction mixture was stirred at room temperature for 30 min and was evaporated at reduced pressure. The resulting reddish gummy residue (62.0 g) was submitted to column chromatography (40–60 μm-silica gel, 450 g, mixtures of hexane/AcOEt). On elution with AcOEt, a colorless gummy mixture (34.5 g) was first isolated, containing alcohols **12b** and **13b** in an approximate ratio of 2:1 (^{13}C NMR), as the main components, which was used as such in the next step. On further elution with AcOEt, pure alcohol **13b** (10.0 g, 30% yield) was isolated, as a crystalline white solid. The analytical sample of **13b** was obtained by recrystallization from AcOEt.

13b: mp 158–159°C. IR (KBr) ν 3518, 2959, 2932, 2898, 1730, 1703, 1458, 1436, 1301, 1250, 1193, 1172, 1146, 1118, 1093, 1022, 989, 949, 923 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, J=7.0 Hz, 3H, CH_2CH_3), 1.41 (ddq, J=14.0 Hz, $J'=J''$=7.0 Hz, 1H) and 1.53 (ddq, J=14.0 Hz, $J'=J''$=7.0 Hz, 1H) (CH_2CH_3), 1.72 (d, J=3.5 Hz, 1H, OH), 1.83 (ddd, J=13.5 Hz, J'=4.5 Hz, J''=2.0 Hz, 1H, 8-H_{endo}), 2.13 (dd, J=14.0 Hz, J'=3.5 Hz, 1H, 4-H_{endo}), 2.19 (d, J=14.0 Hz, 1H, 2-H_{endo}), 2.22 (ddd, J=14.0 Hz, J'=11.0 Hz, J''=3.5 Hz, 1H, 4-H_{exo}), superimposed ca. 2.19–2.26 (m, 1H, 7-H), 2.38 (dd, $J=J'$=13.5 Hz, 1H, 8-H_{exo}), 2.69 (ddd, J=11.0 Hz, $J'=J''$=3.5 Hz, 1H, 5-H), 2.89 (dd, J=14.0 Hz, J'=3.5 Hz, 1H, 2-H_{exo}), 3.76 (s, 3H, $COOCH_3$), 3.88–4.10 (complex signal, 5H, 6-H and OCH_2CH_2O). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 11.9 (CH_3, CH_2CH_3), 23.6 (CH_2, CH_2CH_3), 33.7 (CH, C7), 37.0 (CH_2, C4), 38.8 (CH_2, C8), 40.9 (CH_2, C2), 50.7 (CH, C5), 52.6 (CH_3, $COOCH_3$), 56.4 (C, C1), 64.2 (CH_2) and 65.2 (CH_2) (OCH_2CH_2O), 77.0 (CH, C6), 105.4 (C, C3), 172.5 (C, $COOCH_3$), 210.3 (C, C9). MS, *m/z* (%): 298 ($M^{\cdot+}$, 13), 280 [$(M-H_2O)^{\cdot+}$, 4], 266 [$(M-CH_3OH)^{\cdot+}$, 5], 239 [$(M-COOCH_3)^+$, 15], 227 (10), 214 [$(M-C_5H_8O)^{\cdot+}$, 15], 211 (11), 99 [$(C_5H_7O_2)^+$, 69], 87 (47), 86 [$(C_5H_{10}O)^{\cdot+}$, 100], 55 (85). Anal. calcd for $C_{15}H_{22}O_6$: C, 60.39; H, 7.44. Found: C, 60.34; H, 7.64.

*^{13}C NMR data of **12b**, deduced from the spectrum of the mixture **12b/13b** in the approximate ratio of 2:1 (assignment carried out by comparison with the known 7-methyl-derivative **12a**[20]).* ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 10.9 (CH_3, CH_2CH_3), 23.6 (CH_2, CH_2CH_3), 33.8 (CH_2, C4), 36.0 (CH, C7), 37.3 (CH_2, C8), 40.5 (CH_2, C2), 51.2 (CH, C5), 52.6 (CH_3, $COOCH_3$), 56.5 (C, C1), 64.1 (CH_2) and 65.1 (CH_2) (OCH_2CH_2O), 76.2 (CH, C6), 105.2 (C, C3), 172.3 (C, $COOCH_3$), 208.7 (C, C9).

Methyl 3-ethyl-7,7-ethylenedioxy-9-oxobicyclo[3.3.1]-non-3-ene-1-carboxylate (14b). A solution of **10** (10.0 g, 46.7 mmol) and TMG (1.14 mL, 1.04 g, 9.04 mmol) in anhydrous CH_2Cl_2 (200 mL) was cooled to −78°C and treated dropwise with a solution of α-ethylacrolein (18.3 mL, 15.7 g, 187 mmol) in anhydrous CH_2Cl_2 (50 mL). The reaction mixture was stirred at −78°C for 30 min, was allowed to warm to room temperature for 3 h, and was stirred at room temperature for 2 h. The reaction mixture was evaporated at reduced pressure, the resulting brown gummy residue (15.8 g) was submitted to column chromatography (silica gel, 180 g, AcOEt), to give a colorless gummy residue (13.8 g) which was used as such in the following step.

A solution of the above residue (13.8 g) in anhydrous pyridine (85 mL) was cooled in an ice-water bath, and was treated dropwise with *p*-tolyl chlorothionoformate (8.5 mL, 97% content, 10.4 g, 54.1 mmol). The resulting

reddish mixture was allowed to warm to room temperature, was stirred at room temperature for 3 h, was poured into cold water (250 mL) and was extracted with toluene (5×100 mL). The combined organic extracts were washed successively with 5% aqueous HCl (5×100 mL), water (3×100 mL), and brine (3×100 mL), were dried with anhydrous Na_2SO_4, and evaporated at reduced pressure, to give a brown gummy residue (24.2 g), which was taken up in CH_2Cl_2 (100 mL), treated with active charcoal, and filtered through a short pad of Celite®. Evaporation of the solvent gave a brown oily residue (21.3 g) of the corresponding stereoisomeric mixture of thiocarbonates, which was pyrolyzed without further purification.

The above residue was heated at 250°C/2–4 Torr in two portions (11.4 g+9 90 g) in a rotary microdistillation apparatus. A yellow oil (7.10 g+5.30 g, respectively) distilled, which was taken up altogether in CH_2Cl_2 (100 mL). The resulting solution was washed with 2 N NaOH (3×60 mL), dried with anhydrous Na_2SO_4, and evaporated at reduced pressure to give a yellowish oil (8.20 g), which was submitted to column chromatography (40–60 μm-silica gel, 120 g, mixtures of hexane/AcOEt). On elution with a mixture of hexane/AcOEt in the ratio of 80:20, keto ester **14b** (3.70 g, 28% overall yield from **10**) was isolated as a colorless oil, which solidified on standing.

Note: A similar yield of **14b** (27% overall yield from **10**) was obtained using the above procedure except for the use of DBU as the base catalyst instead of TMG.

The analytical sample of **14b** was obtained by recrystallization from CH_2Cl_2: white crystals, mp 70–71°C. IR (KBr) ν 2967, 2929, 2906, 2880, 2848, 1746, 1715, 1456, 1436, 1350, 1304, 1251, 1230, 1167, 1144, 1110, 1070, 1048, 1002, 952, 934, 836 cm^{-1}. MS, *m/z* (%): 280 ($M^{\cdot+}$, 39), 249 [$(M-CH_3O)^+$, 11], 248 [$(M-CH_3OH)^{\cdot+}$, 37], 221 [$(M-COOCH_3)^+$, 44], 177 [$(M-COOCH_3-C_2H_4O)^+$, 38], 149 [$(M-COOCH_3-C_2H_4O-CO)^+$, 30], 99 [$(C_5H_7O_2)^+$, 68], 91 (100), 87 (93), 86 [$(C_5H_{10}O)^{\cdot+}$, 98], 79 (68), 77 (60), 55 (51). Anal. calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.20. Found: C, 64.44; H, 7.21.

Alkene 14b from alcohol 13b via its mesylate. A stirred solution of alcohol **13b** (2.00 g, 6.71 mmol), Et_3N (9.7 mL, 7.0 g, 70 mmol) and 4-(dimethylamino)pyridine (DMAP) (20.5 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (60 mL) was treated, dropwise, with methanesulfonyl chloride (2.1 mL, 3.1 g, 27 mmol), and the reaction mixture was stirred at room temperature for 6 h. The resulting solution was diluted with CH_2Cl_2 (50 mL), washed with saturated aqueous NH_4Cl (4×50 mL) and dried with anhydrous Na_2SO_4. Evaporation of the solvent at reduced pressure afforded a brown solid residue (1.95 g), which contained the corresponding mesylate impurified with other products (^1H NMR). A solution of this crude mesylate (1.00 g) and DBU (0.85 mL, 0.87 g, 97% content, 5.5 mmol) in anhydrous toluene (45 mL) was heated under reflux for 24 h. After cooling in an ice-water bath, and made acidic with 0.1 N HCl (18 mL), the organic phase was separated and the aqueous one was extracted with CH_2Cl_2 (3×30 mL). The combined organic phase and extracts were dried with anhydrous Na_2SO_4, and evaporated at reduced pressure to give a brown oily residue (930 mg), which was submitted to column chromatography (silica gel, 65 g, mixtures of hexane/AcOEt). On elution with a mixture of hexane/AcOEt in the ratio of 80:20, keto ester **14b** (200 mg, 21% overall yield from alcohol **13b**) was isolated. On elution with a mixture of hexane/AcOEt in the ratio of 30:70, unchanged mesylate was recovered (75 mg).

Methyl (*Z*)-3-ethyl-7,7-ethylenedioxy-9-ethylidenebicyclo[3.3.1]non-3-ene-1-carboxylate, [(*Z*)-15b]. To a stirred mixture of ethyltriphenylphosphonium bromide (26.1 g, 70.3 mmol) and anhydrous THF (260 mL), *n*-BuLi (1.6 M solution in hexane, 37.5 mL, 60.0 mmol) was added dropwise at room temperature over 10 min. The resulting orange suspension was stirred at room temperature for 1.25 h, cooled to 0°C, and treated dropwise for 15 min, with a solution of keto ester **14b** (3.75 g, 13.4 mmol) in anhydrous THF (65 mL). The reaction mixture was allowed to warm to room temperature, stirred at room temperature for 4 h, and poured into water (270 mL). The organic solvents were evaporated at reduced pressure and the resulting aqueous phase was extracted with AcOEt (4×100 mL). The combined organic extracts were washed with brine (100 mL), dried with anhydrous Na_2SO_4, and evaporated at reduced pressure. Column chromatography of the resulting oily residue (14.5 g) (40–60 μm-silica gel, 200 g, mixtures of hexane/AcOEt in the ratio of 80:20) afforded a mixture of esters (*Z*)-**15b** and (*E*)-**15b** in an approximate ratio of 85:15 (^1H NMR) (3.50 g, 89% yield), as a white solid. Purification of (*Z*)-**15b** from this diastereomeric mixture by a second column chromatography under the above conditions or by distillation at 110°C/1 Torr or 160°C/2 Torr was fruitless.

*Pure (Z)-**15b** by partial hydrolysis of the mixture of (Z)-**15b**/(E)-**15b** in the ratio of 85:15.* A mixture of (*Z*)-**15b**/(*E*)-**15b** in the ratio of 85:15 (169 mg, 0.58 mmol), 20% aqueous NaOH (12 mL, 60 mmol), THF (12 mL) and MeOH (12 mL) was stirred under reflux for 6 h. The organic solvents were evaporated in vacuo and the remaining aqueous mixture was extracted with CH_2Cl_2 (2×25 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and evaporated at reduced pressure to afford pure (*Z*)-**15b** [130 mg, 90% of recovered (*Z*)-**15b**], as a colorless oil. The analytical sample of (*Z*)-**15b** was obtained by distillation at 150°C/1 Torr. IR (NaCl) ν 2962, 2938, 2920, 2874, 1729, 1458, 1434, 1237, 1200, 1167, 1148, 1129, 1100, 1055, 996, 951, 925, 834, 702 cm^{-1}. MS, *m/z* (%): 292 ($M^{\cdot+}$, 0.4), 248 [$(M-C_2H_4O)^{\cdot+}$, 2], 216 [$(M-C_2H_4O-CH_3OH)^{\cdot+}$, 27], 191 [$(M-C_5H_9O_2)^+$, 40], 189 [$(M-C_5H_{11}O_2)^+$, 27], 159 [$(M-C_5H_9O_2-CH_3OH)^+$, 100], 131 [$(M-C_5H_9O_2-HCOOCH_3)^+$, 37], 119 [$(C_9H_{11})^+$, 71], 91 (62). Anal. calcd for $C_{17}H_{24}O_4$: C, 69.83; H, 8.28. Found: C, 70.07; H, 8.44.

Methyl (*E*)-3-ethyl-7,7-ethylenedioxy-9-ethylidenebicyclo[3.3.1]non-3-ene-1-carboxylate [(*E*)-15b]. A solution of a mixture of (*Z*)-**15b**/(*E*)-**15b** in the ratio of 85:15 (640 mg, 2.19 mmol), AIBN (256 mg, 1.56 mmol) and thiophenol (0.35 mL, 376 mg, 3.41 mmol) in anhydrous toluene (7 mL) was heated at 85°C for 22 h. The mixture was concentrated in vacuo and the resulting residue was taken up in CH_2Cl_2 (20 mL). The organic solution was washed successively with 2 N NaOH (2×15 mL) and brine

(30 mL), dried with anhydrous Na_2SO_4, and evaporated at reduced pressure to give a yellow oil (780 mg), which was submitted to column chromatography (silica gel, 50 g, mixtures of hexane/AcOEt). On elution with a mixture of hexane/AcOEt in the ratio of 80:20, a mixture of esters (*E*)-**15b** and (*Z*)-**15b** in an approximate ratio of 95:5 (^{1}H NMR) (550 mg, 86% yield) was obtained. An analytical sample of the above mixture was obtained by distillation at 125°C/1.5 Torr. IR (NaCl) ν 2963, 2928, 2901, 2878, 1729, 1457, 1433, 1238, 1158, 1130, 1092, 1052, 996, 951, 927, 839, 703 cm^{-1}. MS, *m/z* (%): 292 ($M^{\cdot+}$, 10), 233 [$(M-COOCH_3)^+$, 23], 231 (10), 230 (11), 224 [$(M-C_5H_8)^{\cdot+}$, 24], 191 [$(M-C_5H_9O_2)^+$, 68], 159 [$(M-C_5H_9O_2-CH_3OH)^+$, 95], 131 [$(M-C_5H_9O_2-HCOOCH_3)^+$, 40], 119 [$(C_9H_{11})^+$, 52], 91 (94), 87 [$(C_4H_7O_2)^+$, 100], 86 (76). Anal. calcd for $C_{17}H_{24}O_4$: C, 69.83; H, 8.28. Found: C, 69.35; H, 8.37.

(*E*)-3-Ethyl-9-ethylidene-7-oxobicyclo[3.3.1]non-3-ene-1-carboxylic acid (16b). A mixture (*E*)-**15b**/(*Z*)-**15b** in the ratio of 95:5 (580 mg, 1.99 mmol), 20% aqueous NaOH (38 mL, 0.19 mol), THF (38 mL) and MeOH (38 mL) was stirred under reflux for 48 h. The organic solvents were evaporated in vacuo and the remaining aqueous mixture was washed with CH_2Cl_2 (2×50 mL), made acidic with 5 N HCl (50 mL), and extracted with CH_2Cl_2 (3×30 mL). The combined organic extracts were washed with brine (2×40 mL), dried with anhydrous Na_2SO_4, and evaporated at reduced pressure. The resulting yellow oily residue (0.48 g) was taken up in dioxane (10 mL), treated with 2 N HCl (10 mL), and the mixture was stirred at room temperature for 4 h. The organic solvent was evaporated in vacuo and the remaining aqueous mixture was extracted with CH_2Cl_2 (3×40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, and evaporated at reduced pressure to give a yellowish solid residue (455 mg), which was submitted to column chromatography (silica gel, 60 g, mixtures of hexane/AcOEt). On elution with a mixture of hexane/AcOEt in the ratio of 50:50, pure (*E*)-**16b** (350 mg, 75% yield) was isolated as a white solid. The analytical sample of **16b** was obtained by sublimation at 90°C/1 Torr, mp 166–168°C. IR (KBr) ν 3650–2300 (max. at 3437, 2955, 2920, 2862, 2756, 2591), 1728, 1680, 1442, 1381, 1327, 1225, 1164, 1088, 1037, 943, 829, 786, 712, 658 cm^{-1}. MS, *m/z* (%): 234 ($M^{\cdot+}$, 5), 216 [$(M-H_2O)^{\cdot+}$, 11], 189 [$(M-COOH)^+$, 31], 188 [$(M-HCOOH)^{\cdot+}$, 11], 177 [$(M-C_3H_5O)^+$, 45], 159 [$(M-C_3H_5O-H_2O)^+$, 100], 147 (44), 131 [$(M-C_3H_5O-HCOOH)^+$, 36], 119 [$(C_9H_{11})^+$, 81], 117 [$(C_9H_9)^+$, 33], 105 [$(C_8H_9)^+$, 39], 91 (66). Anal. calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.75. Found: C, 71.45; H, 7.89.

Methyl (*E*)-*N*-{3-ethyl-9-ethylidene-7-oxobicyclo[3.3.1]non-3-en-1-yl}carbamate (17b). A solution of keto acid **16b** (860 mg, 3.68 mmol), Et_3N (0.52 mL, 378 mg, 3.73 mmol) and diphenylphosphoryl azide (0.86 mL, 1.09 g, 97% content, 3.87 mmol) in anhydrous chlorobenzene (17 mL) was heated at 90°C for 3.5 h. Anhydrous MeOH (25 mL) was added and the resulting mixture was heated under reflux for 17 h. The solvent was evaporated at reduced pressure to give a residue (1.90 g), which was submitted to column chromatography (silica gel, 85 g, CH_2Cl_2 and then mixtures of CH_2Cl_2/AcOEt in the ratio of 75:25). On elution with CH_2Cl_2/AcOEt in the ratio of 75:25, keto carbamate **17b** (800 mg, 83% yield) was isolated, as a white solid. The analytical sample of **17b** was obtained by recrystallization from a mixture of CH_2Cl_2/hexane in the ratio of 1:3, mp 80–82°C. IR (KBr) ν 3317, 3058, 2964, 2924, 2889, 1702, 1542, 1436, 1381, 1320, 1270, 1221, 1193, 1079, 1039, 956, 864, 780, 701 cm^{-1}. MS, *m/z* (%): 263 ($M^{\cdot+}$, 3), 248 [$(M-CH_3)^+$, 2], 234 [$(M-CH_2CH_3)^+$, 4], 231 [$(M-CH_3OH)^{\cdot+}$, 8], 206 [$(M-C_3H_5O)^+$, 100], 188 [$(M-NH_2COOCH_3)^{\cdot+}$, 35], 174 [$(M-C_3H_5O-CH_3OH)^+$, 98], 146 [$(M-C_3H_5O-HCOOCH_3)^+$, 73], 131 [$(M-C_3H_5O-NH_2COOCH_3)^+$, 57], 117 [$(C_9H_9)^+$, 31], 91 (55). Anal. calcd for $C_{15}H_{21}NO_3$: C, 68.41; H, 8.04; N, 5.32. Found: C, 68.41; H, 8.17; N, 5.41.

(*E*)-11-Ethylidene-5,6,9,10-tetrahydro-5-(methoxycarbonylamino)-7-methyl-5,9-methano-1*H*-cycloocta[*b*]-pyridin-2-one (18a), and (*E*)-11-ethylidene-5,8,9,10-tetrahydro-9-(methoxycarbonylamino)-7-methyl-5,9-methano-1*H*-cycloocta[*b*]pyridin-2-one (19a). A mixture of keto carbamate **17a** (1.90 g, 7.63 mmol), pyrrolidine (0.80 mL, 0.69 g, 9.7 mmol), 4 Å molecular sieves (5 g) and anhydrous benzene (95 mL) was heated under reflux for 5 h. The mixture was allowed to cool to room temperature and was filtered under argon. The filtrate was treated with freshly prepared propiolamide[24] (1.40 g, 20.3 mmol) and the reaction mixture was heated under reflux for 21 h. The resulting orange suspension was allowed to cool to room temperature and filtered. The filtrate was evaporated at reduced pressure to give a reddish residue (2.80 g), which was submitted to column chromatography (40–60 μm-silica gel, 350 g, mixtures of hexane/AcOEt and then mixtures of AcOEt/MeOH). On elution with a mixture of hexane/AcOEt in the ratio of 30:70, starting keto carbamate **17a** (600 mg) was recovered. On elution with AcOEt, **19a** slightly contaminated with **18a** (407 mg, 18% yield, 26% yield taking into account the recovered **17a**) was isolated. On elution with a mixture of AcOEt/MeOH in the ratio of 90:10, pure **18a** (620 mg, 27% yield, 40% yield taking into account the recovered **17a**) was isolated. The analytical samples of pyridones **18a** and **19a** were obtained by recrystallization from MeOH and from a mixture of AcOEt/MeOH in the ratio of 1:2, respectively.

18a: mp 284–284.5°C (dec.). IR (KBr) ν 3321, 3248, 3001, 2932, 1714, 1654, 1619, 1555, 1537, 1459, 1308, 1273, 1249, 1180, 1107, 1070, 1040, 933, 836, 752 cm^{-1}.

19a: mp 266–267°C (dec.). IR (KBr) ν 3279, 3236, 3025, 2931, 1724, 1651, 1617, 1555, 1457, 1256, 1223, 1192, 1109, 1068, 1045, 830, 755, 663, 636 cm^{-1}. MS, *m/z* (%): 300 ($M^{\cdot+}$, 27), 299 [$(M-H)^+$, 10], 285 [$(M-CH_3)^+$, 4], 268 [$(M-CH_3OH)^{\cdot+}$, 4], 253 [$(M-H-HCOOH)^+$, 10], 239 [$(M-H-HCOOCH_3)^+$, 8], 226 (34), 225 [$(M-NH_2COOCH_3)^{\cdot+}$, 100], 224 (49), 210 [$(M-NH_2COOCH_3-CH_3)^+$, 88]. Anal. calcd for $C_{17}H_{20}N_2O_3\cdot 1/5H_2O$: C, 67.17; H, 6.77; N, 9.22. Found: C, 67.18; H, 6.96; N, 9.18.

(*E*)-7-Ethyl-11-ethylidene-5,6,9,10-tetrahydro-5-(methoxycarbonylamino)-5,9-methano-1*H*-cycloocta[*b*]pyridin-2-one (18b), and (*E*)-7-ethyl-11-ethylidene-5,8,9,10-tetrahydro-9-(methoxycarbonylamino)-5,9-methano-1*H*-cycloocta[*b*]pyridin-2-one (19b). A mixture of keto carbamate

17b (673 mg, 2.56 mmol), pyrrolidine (0.27 mL, 232 mg, 3.26 mmol), 4 Å molecular sieves (1.50 g) and anhydrous benzene (30 mL) was heated under reflux for 5 h. The mixture was allowed to cool to room temperature and was filtered under argon. The filtrate was treated with freshly prepared propiolamide[24] (530 mg, 7.68 mmol) and the reaction mixture was heated under reflux for 15 h. The resulting orange suspension was allowed to cool to room temperature, was diluted with MeOH (120 mL) and was filtered. The filtrate was evaporated at reduced pressure to give an orange residue (1.40 g), which was taken up in AcOEt (450 mL) and was extracted with 1 N NaOH (6×100 mL). The combined aqueous extracts were neutralized with 0.75 N HCl (ca. 800 mL) and extracted successively with CH_2Cl_2 (5×100 mL) and AcOEt (5×100 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (2×50 mL), dried with anhydrous Na_2SO_4, and evaporated at reduced pressure, to give a yellow-green solid residue (520 mg), which was submitted to column chromatography (40–60 μm-silica gel, 52 g, mixtures of hexane/AcOEt, and then AcOEt/MeOH). On elution with a mixture of AcOEt/MeOH in the ratio of 97:3, pure **19b** (66 mg), a mixture of **19b** and **18b** in the ratio of 2:5 (^{1}H NMR) (72 mg), and pure **18b** (99 mg) were consecutively obtained. The mixture **19b/18b** in the ratio of 2:5 (72 mg) was submitted again to column chromatography (silica gel, 14 g) under the same conditions. On elution with a mixture of AcOEt/MeOH in the ratio of 98:2, pure **19b** (10 mg) and pure **18b** (50 mg), were consecutively eluted.

On the other hand, the initial AcOEt solution was dried with anhydrous Na_2SO_4 and evaporated at reduced pressure to give an orange residue (1.40 g), which was submitted to column chromatography (silica gel, 100 g, mixtures of hexane/AcOEt and then, mixtures of AcOEt/MeOH). On elution with a mixture of hexane/AcOEt in the ratio of 75:25, starting keto carbamate **17b** (130 mg) was recovered. On elution with a mixture of AcOEt/MeOH in the ratio of 95:5, a mixture of pyridones **19b/18b** in an approximate ratio of 2:3 (150 mg) was obtained. This mixture was again submitted to column chromatography (silica gel, 15 g) under the same conditions. On elution with a mixture of AcOEt/MeOH in the ratio of 97:3, pure (*E*)-**19b** (50 mg), a mixture **19b/18b** in a ratio of 1:4 (^{1}H NMR) (40 mg), and pure **18b** (30 mg) were consecutively eluted [22% total yield of **18b** and 16% total yield of **19b**, 28 and 19%, respectively, taking into account the amount of recovered **17b**]. The analytical samples of pyridones **18b** and **19b** were obtained by recrystallization from a mixture of AcOEt/MeOH in the ratio of 8:1 and from AcOEt, respectively.

18b: mp 237–239°C. IR (KBr) ν 3591, 3514, 3360, 3120, 2971, 2956, 2880, 2832, 2796, 1717, 1650, 1619, 1553, 1523, 1458, 1308, 1251, 1186, 1111, 1047, 959, 934, 833, 779, 640, 619 cm^{-1}. MS, *m/z* (%): 315 [$(M+H)^+$, 7], 314 ($M^{\cdot+}$, 31), 313 [$(M-H)^+$, 2], 299 [$(M-CH_3)^+$, 6], 285 [$(M-CH_2CH_3)^+$, 15], 282 [$(M-CH_3OH)^{\cdot+}$, 7], 267 [$(M-CH_3OH-CH_3)^+$, 5], 253 [$(M-H-HCOOCH_3)^+$, 35], 239 [$(M-NH_2COOCH_3)^{\cdot+}$, 66], 224 [$(M-NH_2COOCH_3-CH_3)^+$, 53], 210 [$(M-NH_2COOCH_3-CH_2CH_3)^+$, 100]. Anal. calcd for $C_{18}H_{22}N_2O_3.2/3H_2O$: C, 66.23; H, 7.21; N, 8.58. Found: C, 66.23; H, 7.02; N, 8.49.

19b: mp 236–238°C (dec.). IR (KBr) ν 3428, 3317, 3235, 3045, 2962, 2927, 1707, 1652, 1619, 1557, 1526, 1458, 1276, 1250, 1188, 1107, 1043, 827, 779, 639 cm^{-1}. MS, *m/z* (%): 315 [$(M+H)^+$, 5], 314 ($M^{\cdot+}$, 25), 313 [$(M-H)^+$, 10], 299 [$(M-CH_3)^+$, 3], 285 [$(M-CH_2CH_3)^+$, 5], 282 [$(M-CH_3OH)^{\cdot+}$, 4], 281 (3), 267 [$(M-CH_3OH-CH_3)^+$, 5], 240 (31), 239 [$(M-NH_2COOCH_3)^{\cdot+}$, 100], 238 (43), 224 [$(M-NH_2COOCH_3-CH_3)^+$, 55], 210 [$(M-NH_2COOCH_3-CH_2CH_3)^+$, 70]. Anal. calcd for $C_{18}H_{22}N_2O_3.1/2H_2O$: C, 66.85; H, 7.17; N, 8.66. Found: C, 66.86; H, 7.08; N, 8.57.

(*E*)-9-Amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methano-1*H*-cycloocta[*b*]pyridin-2-one (20a). It was prepared in a similar manner to that described for compound **2**, starting from a suspension of carbamate **19a** (105 mg, 0.35 mmol) in $CHCl_3$ (13 mL), TMSI (0.50 mL, 0.70 g, 3.5 mmol), and MeOH (13 mL). The resulting brown residue (320 mg) was submitted to column chromatography (ammonia-saturated silica gel, 25 g, mixtures of $CHCl_3$/MeOH). On elution with a mixture of $CHCl_3$/MeOH in the ratio of 85:15, impure amine **20a** was obtained (230 mg) as a white solid. This product was again submitted to column chromatography (ammonia-saturated silica gel, 20 g, mixtures of hexane/AcOEt, and then mixtures of AcOEt/MeOH). On elution with a mixture of AcOEt/MeOH in the ratio of 90:10, pure **20a** (85 mg, quantitative yield) was isolated as a white solid. The analytical sample of **20a** was obtained by recrystallization from MeOH, mp 187–193°C (dec.). IR (KBr) ν 3507, 3353, 3315, 3116, 2954, 2930, 2889, 2839, 2825, 2504, 2452, 2348, 2337, 2184, 2138, 1650, 1559, 1457, 1428, 1407, 1382, 1204, 1178, 1117, 972, 941, 833, 790, 656, 631, 608 cm^{-1}. MS, *m/z* (%): 243 [$(M+H)^+$, 17], 242 ($M^{\cdot+}$, 81), 241 [$(M-H)^+$, 35], 228 (19), 227 [$(M-CH_3)^+$, 100], 226 [$(M-NH_2)^+$, 13], 225 [$(M-NH_3)^{\cdot+}$, 16], 214 [$(M-CO)^{\cdot+}$, 44], 213 [$(M-H-CO)^+$, 61], 212 (30), 210 (35). Exact mass calcd for $C_{15}H_{18}N_2O$ 242.1419, obsd 242.1412.

(*E*)-9-Amino-7-ethyl-11-ethylidene-5,6,9,10-tetrahydro-5,9-methano-1*H*-cycloocta[*b*]pyridin-2-one (20b). It was prepared in a similar manner to that described for compound **2**, starting from a suspension of carbamate **19b** (86 mg, 0.27 mmol) in $CHCl_3$ (10 mL), TMSI (0.39 mL, 0.55 g, 2.7 mmol), and MeOH (10 mL). The resulting brown residue (160 mg) was submitted to column chromatography (40–60 μm-silica gel, 8 g, mixtures of $CHCl_3$/MeOH). On elution with a mixture of $CHCl_3$/MeOH in the ratio of 96:4, impure amine **20b** was obtained (134 mg) as a yellowish solid. This product was again submitted to column chromatography (40–60 μm-silica gel, 27 g, mixtures of hexane/AcOEt, and then mixtures of AcOEt/MeOH). On elution with a mixture of AcOEt/MeOH in the ratio of 80:20, almost pure **20b** (59 mg, 85% yield) was isolated as a white solid. The analytical sample of **20b** was obtained after twofold chromatography (silica gel) under the same conditions followed by recrystallization from a mixture of hexane/AcOEt in the ratio of 2:1, mp 190–191°C (dec.). IR (KBr) ν 3426, 3359, 3256, 3097, 2954, 2930, 2883, 1653, 1622, 1553, 1458, 1409, 1384, 1205, 1111, 938, 824, 707, 655, 626 cm^{-1}. MS, *m/z* (%): 257 [$(M+H)^+$, 13], 256 ($M^{\cdot+}$, 56), 255 [$(M-H)^+$, 21], 242 (10), 241 [$(M-CH_3)^+$, 47], 239 [$(M-NH_3)^{\cdot+}$, 12], 228 (38), 227 [$(M-CH_2CH_3)^+$, 100],

Table 8. Experimental data of the X-ray crystal structure determination of **13b**

Molecular formula	$C_{15}H_{22}O_6$	$F(000)$	1280
Molecular mass	298.33	d (calcd) [Mg m^{-3}]	1.331
Temperature	293(2)K	Size of crystal [mm]	0.1×0.1×0.2
Crystal system	Orthorhombic	Measured reflections	5778
Space group	*Pcab*	Independent reflections	3415
Cell parameters	[a]	Observed reflections	2663
a [Å]	10.147(6)	μ(Mo-Kα) [mm^{-1}][b]	0.100
b [Å]	14.550(3)	R	0.058
c [Å]	20.172(4)	Rw	0.121
α [°]	90	$\Delta\rho_{max}$[c] (eÅ^{-3})	0.299
β [°]	90	$\Delta\rho_{min}$[d] (eÅ^{-3})	−0.175
γ [°]	90	Refined parameters	279
V [Å^3]	2978(2)	Max. shift/e.s.d.	0.00
Z	8		

[a] Determined by automatic centering of 25 reflections ($12 \leq \theta \leq 21°$).
[b] μ(Mo-Kα), Linear absorption coefficient. Radiation Mo-Kα (λ=0.71069 Å).
[c] Maximum peaks in final difference synthesis.
[d] Minimum peaks in final difference synthesis.

212 (31). Exact mass calcd for $C_{16}H_{20}N_2O$ 256.1576, obsd 256.1567.

Attempted synthesis of (*E*)-15a from keto ester 14a: Obtention of 7,7-ethylenedioxy-5-(methoxycarbonyl)-3-methylcyclooct-2-enecarboxylic acid (22). A solution of lithium diisopropylamide (LDA) (2 M solution in heptane/THF/ethylbenzene, 1.90 mL, 3.80 mmol) in anhydrous THF (25 mL) was cooled to −78°C. To this stirred solution was added dropwise over 5 min, *S*-phenyl thiopropionate (0.58 mL, 0.63 g, 3.80 mmol). The mixture was stirred at −78°C for 30 min, and was treated dropwise with a solution of keto ester **14a** (1.00 g, 3.76 mmol) in anhydrous THF (10 mL). The reaction mixture was stirred at −78°C for 30 min, was allowed to warm slowly to 0°C during 1.5 h, and was treated with saturated aqueous NH_4Cl (20 mL). To this mixture, water (50 mL) and diethyl ether (50 mL) were added. The aqueous phase was separated and the organic one was washed with 10% aqueous K_2CO_3 (2×50 mL) and brine (50 mL), was dried with anhydrous Na_2SO_4, and was evaporated at reduced pressure, to give starting keto ester **14a** (90 mg). The initial aqueous phase was treated with cold 2 N HCl (20 mL) and extracted with CH_2Cl_2 (3×50 mL). The combined organic extracts were washed with brine (2×40 mL), dried with anhydrous Na_2SO_4, and evaporated at reduced pressure, to give a mixture of diastereomers of **22** in an approximate ratio of 55:45 (^{1}H NMR) (0.70 g, 69% yield) as a yellowish oil. The analytical sample was obtained by column chromatography (silica gel, 40 g, mixtures of hexane/AcOEt) of a portion of this crude (200 mg). On elution with a mixture of hexane/AcOEt in the ratio of 55:45, pure diastereomeric mixture **22** (118 mg) was isolated.

*Spectroscopic and analytical data of the diastereomeric mixture **22**.* IR (NaCl) ν 3500–2500 (max. at 3464, 3190, 2642), 2949, 2883, 1733, 1708, 1437, 1345, 1288, 1200, 1170, 1129, 1096, 1047, 1001, 949, 921, 896, 827, 732 cm^{-1}. MS, *m*/*z* (%): 284 ($M^{\cdot+}$, 1), 266 [$(M-H_2O)^{\cdot+}$, 6], 253 [$(M-CH_3O)^+$, 1], 252 [$(M-CH_3OH)^{\cdot+}$, 1], 239 [$(M-COOH)^+$, 4], 238 [$(M-HCOOH)^{\cdot+}$, 3], 225 [$(M-COOCH_3)^+$, 3], 222 [$(M-H_2O-C_2H_4O)^{\cdot+}$, 4], 157 [$(C_7H_9O_4)^+$, 22], 86 [$(C_4H_6O_2)^{\cdot+}$, 100], 55 [$(C_3H_3O)^+$, 22]. Anal. calcd for $C_{14}H_{20}O_6 \cdot H_2O$: C, 55.62; H, 7.34. Found: C, 55.83; H, 7.69.

*NMR data of the main stereoisomer deduced from the spectra of the mixture **22**.* ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.64 (d, J=1.0 Hz, 3H, 3-CH_3), 1.82 (dd, J=13.5 Hz, J'=12.5 Hz, 1H, 8-H_a), 2.00–2.13 (complex signal, 3H, 6-H_a, 6-H_b and 8-H_b), superimposed in part 2.40 (dd, J=13.5 Hz, J'=1.5 Hz, 1H, 4-H_a), 2.77 (dd, J=13.5 Hz, J'=8.0 Hz, 1H, 4-H_b), superimposed in part 2.82 (m, 1H, 5-H), 3.40 (broad dd, J=11.5 Hz, J'=8.0 Hz, 1H, 1-H), 3.65 (s, 3H, $COOCH_3$), 3.64–3.96 (complex signal, 4H, OCH_2CH_2O), 5.64 (dm, J=8.0 Hz, 1H, 2-H), 7.40–10.40 (broad signal, 1H, COOH). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 24.3 (CH_3, 3-CH_3), 32.1 (CH_2, C4), 36.5 (CH_2, C6), 38.55 (CH, C5), 40.23 (CH, C1), 42.5 (CH_2, C8), 51.82 (CH_3, $COOCH_3$), 63.7 (CH_2) and 65.1 (CH_2) (OCH_2CH_2O), 109.1 (C, C7), 124.0 (CH, C2), 135.6 (C, C3), 176.0 (C, $COOCH_3$), 180.43 (C, COOH).

*NMR data of the minor stereoisomer deduced from the spectra of the mixture **22**.* ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.72 (d, J=1.0 Hz, 3H, 3-CH_3), 1.78 (dd, J=13.5 Hz, J'=12.5 Hz, 1H, 8-H_a), 2.00–2.13 (complex signal, 2H, 6-H_a and 8-H_b), 2.24 (dd, J=13.0 Hz, J'=5.0 Hz, 1H, 4-H_a), superimposed in part 2.38 (dd, J=14.0 Hz, J'=3.5 Hz, 1H, 6-H_b), 2.53 (dddd, J=12.5 Hz, J'=J''=5.0 Hz, J'''=3.5 Hz, 1H, 5-H), 2.73 (dd, J=J'=13.0 Hz, 1H, 4-H_b), 3.53 (ddd, J=12.5 Hz, J'=8.5 Hz, J''=2.0 Hz, 1H, 1-H), 3.68 (s, 3H, $COOCH_3$), 3.64–3.96 (complex signal, 4H, OCH_2CH_2O), 5.59 (boad d, J=8.5 Hz, 1H, 2-H), 7.40–10.40 (broad signal, 1H, COOH). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 23.1 (CH_3, 3-CH_3), 31.6 (CH_2, C4), 37.5 (CH_2, C6), 38.47 (CH, C5), 40.19 (CH, C1), 43.3 (CH_2, C8), 51.75 (CH_3, $COOCH_3$), 63.9 (CH_2) and 65.2 (CH_2) (OCH_2CH_2O), 109.5 (C, C7), 123.3 (CH, C2), 137.2 (C, C3), 175.2 (C, $COOCH_3$), 180.37 (C, COOH).

X-Ray crystal-structure determination of 13b[38]

A prismatic crystal was selected and mounted on a Enraf-Nonius CAD4 four-circle diffractometer. Unit-cell parameters were determined by automatic centering of 25

reflections ($12<\theta<21°$) and refined by the least-squares method. Intensities were collected with graphite-monochromatized Mo-Kα radiation, using $\omega/2\theta$ scan technique (Table 8). 5778 reflections were measured in the range $2.02\leq\theta\leq30.07$, 3415 of which were non-equivalent by symmetry [R_{int} (on I)=0.052]. 2663 reflections were assumed as observed by applying the condition $I>2\sigma(I)$. Three reflections were measured every two hours as orientation and intensity control; significant intensity decay was not observed. Lorentz polarization but no absorption corrections were made. The structure was solved by Direct methods, using the SHELXS computer program[39] and refined by the full-matrix least-squares method with the SHELX-93 computer program[40] using 3365 reflections (very negative intensities were not assumed). The function minimized was $\sum w(|F_o|^2-|F_c|^2)^2$, where $w=[\sigma^2(I)+(0.0585P)^2+0.2945P]^{-1}$, and $P=(|F_o|^2+2|F_c|^2)/3$. f, f' and f'' were taken from International Tables of X-ray Crystallography.[41] All H atoms were located from a difference synthesis and refined with an overall isotropic temperature factor. Goodness of fit on F^2=1.137 for all observed reflections. Mean shift/e.s.d.=0.00.

Determination of AChE inhibitory activity

AChE inhibitory activity was evaluated spectrophotometrically at 25°C by the method of Ellman,[29] using AChE from bovine erythrocytes and acetylthiocholine iodide (0.53 mM) as substrate. The reaction took place in a final volume of 3 mL of 0.1 M phosphate-buffered solution pH 8.0, containing 0.025 units of AChE and 333 μM 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB) solution used to produce the yellow anion of 5-thio-2-nitrobenzoic acid. Inhibition curves with different derivatives were performed in triplicate by incubating with at least 12 concentrations of inhibitor for 15 min. One triplicate sample without inhibitor was always present to yield the 100% of AChE activity. The reaction was stopped by the addition of 100 μL 1 mM eserine, and the color production was measured at 412 nm. The drug concentration producing 50% of AChE activity inhibition (IC_{50}) was calculated. Results are expressed as mean±S.E.M. of at least four experiments. DTNB, acetylthiocholine and the enzyme were purchased from Sigma and eserine from Fluka.

Acknowledgements

A fellowship from Agencia Española de Cooperación Internacional (Instituto de Cooperación con el Mundo Árabe, Mediterráneo y Países en Desarrollo) to R. El Achab and financial support from Fundació La Marató de TV3 (project 3004/97) are gratefully acknowledged. We also thank the Serveis Científico-Tècnics of the University of Barcelona and particularly Dr M. Feliz and Dr A. Linares for recording the NMR spectra, Ms P. Domènech from the Centro de Investigación y Desarrollo (C.I.D.) (Barcelona, Spain) for carrying out the elemental analyses, and Prof. Dr A. Kozikowski of the Georgetown University Medical Center, Washington, USA, for a generous gift of a sample of natural (−)-huperzine A.

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