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SYNTHESIS AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF FLUORINATED ANALOGUES OF HUPERZINE A

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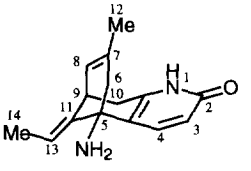
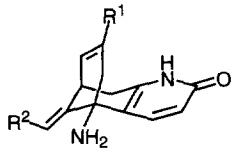
Abstract : Three novel fluorinated analogues of huperzine A (**1**), (±)-12,12,12-trifluorohuperzine A (**2**), (±)-14,14,14-trifluorohuperzine A (**3**), and (±)-12,12,12,14,14,14-hexafluorohuperzine A (**4**), have been synthesized by employing $TMSCF_3$ as a trifluoromethylating agent. These analogues were found to still retain anti-acetylcholinesterase activity. Copyright © 1996 Elsevier Science Ltd

(–)-Huperzine A (**1**) isolated from *Huperzia serrata* (Thunb.) Trev., a Chinese folk medicine, has been shown to be a powerful selective inhibitor of acetylcholinesterase (AChE) (**Figure 1**).[3-5] The use of **1** to increase the level of the neurotransmitter acetylcholine in the central nervous system is considered to be one of potential therapeutic approaches for the treatment of Alzheimer's desease.[6] A number of analogues of **1** have been synthesized and their anti-AChE activity has been examined to date.[7] However, to the best of our knowledge, there have been no reports on the synthesis of the fluorinated analogues of **1**. Therefore, we became interested in designing and synthesizing three novel fluorinated analogues of **1**, (±)-12,12,12-trifluorohuperzine A (**2**), (±)-14,14,14-trifluorohuperzine A (**3**), and (±)-12,12,12,14,14,14-hexafluorohuperzine A (**4**), in order to evaluate their biological properties. We wish to report here the synthesis of the fluorinated analogues **2** - **4** as well as their AChE inhibitory activity. The explored synthetic pathway to **2** - **4** are based upon Kozikowski's method developed in the synthesis of **1** and its analogues.[4a,7e] The key feature of our synthesis consists of the direct introduction of trifluoromethyl group employing Ruppert's reagent[8] ($TMSCF_3$, (trifluoromethyl)trimethylsilane) (**7** → **8**, **17** → **18**, and **30** → **31**).

Figure 1. Structures of Huperzine A (**1**) and Its Fluorinated Analogues **2**, **3**, and **4**



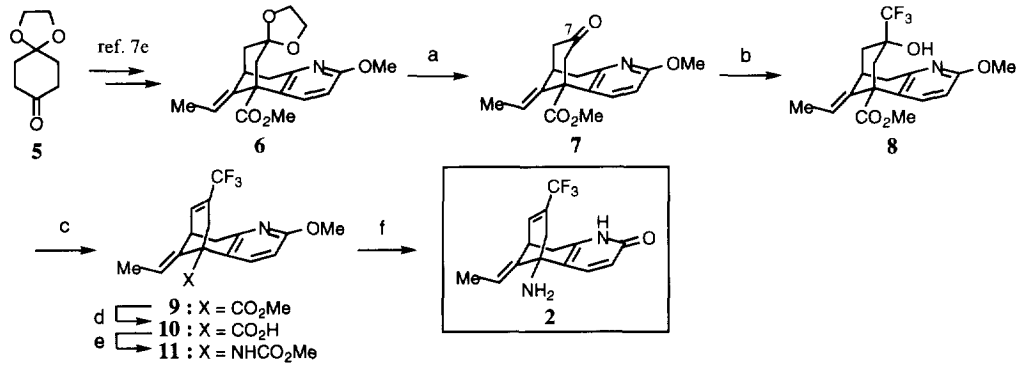
huperzine A (**1**)



(±)-12,12,12-trifluorohuperzine (**2**) : $R^1 = CF_3$, R^2 = Me
(±)-14,14,14-trifluorohuperzine (**3**) : R^1 = Me, $R^2 = CF_3$
(±)-12,12,12,14,14,14-hexafluorohuperzine (**4**) : $R^1 = R^2 = CF_3$

At first, we pursued the synthesis of (±)-12,12,12-trifluorohuperzine A (**2**) as shown in **Scheme 1**. Thus, the known ketal **6**[7e] prepared from commercially available 1,4-cyclohexanedione monoethylene ketal (**5**), was subjected to acid hydrolysis to provide ketone **7**. The crucial introduction of a trifluoromethyl group was

Scheme 1. Synthesis of (±)-12,12,12-Trifluorohuperzine A (**2**)



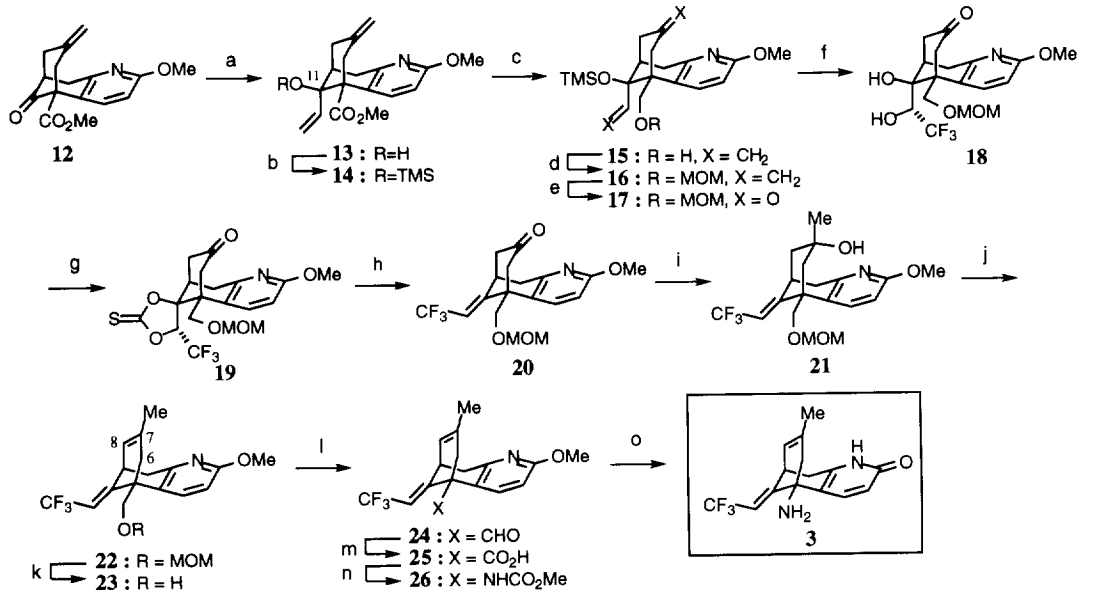
reagents and conditions : a) aq HCl, 2-propanol, 70°C, 90% b) $TMSCF_3$, TBAF, THF, rt ; TBAF, 50% c) $SOCl_2$, pyridine, 0°C, 61% d) aq NaOH, THF-MeOH, reflux, 89% e) DPPA, Et_3N, toluene, 85°C ; MeOH, reflux, 58% f) TMSI, $CHCl_3$, reflux ; MeOH, reflux, 93%

carried out by treating **7** with $TMSCF_3$ in the presence of tetra-*n*-butylammonium fluoride (TBAF),[8] leading to the trifluoromethylated product **8** in 50% yield. Dehydration of **8** with thionyl chloride in pyridine furnished olefin **9** as a single regioisomer in 61% yield. Olefin **9** was then converted to the corresponding methyl carbamate **11** via a sequence of reactions involving saponification, Curtius rearrangement of the liberated acid **10**, and methanolysis of the resulting isocyanate. Finally, deprotection of **11** with iodotrimethylsilane resulted in the formation of **2**, mp 264-265 °C.

Next, the synthesis of (±)-14,14,14-trifluorohuperzine A (**3**) starting from the known keto ester **12**[7e] was investigated as shown in **Scheme 2**. Thus, treatment of **12** with vinylmagnesium bromide provided alcohol **13**, whose hydroxy group was then protected to give trimethylsilyl (TMS) ether **14** as a single stereoisomer in 51% overall yield from **12**. The stereochemistry at the C-11 position of **14** was determined by NOESY experiments. Sequential reduction of **14** with diisobutylaluminium hydride (DIBAL), protection of the resulting alcohol **15** as its methoxymethyl (MOM) ether, and ozonolysis of the two terminal olefins in the MOM ether **16**, gave rise to keto aldehyde **17** in 39% overall yield from **14**. The critical trifluoromethylation of **17** took place in a highly chemo- and stereoselective manner to afford vicinal diol **18** as a single isomer in 81% yield. After treatment of **18** with 1,1'-thiocarbonyldiimidazole, the resulting thiocarbonate **19** was subjected to stereospecific olefin formation,[9] affording the desired *E*-olefin **20** (73%, 2 steps). The configuration of the olefinic double bond in **20** was determined by NOE studies. Treatment of **20** with methyllithium and subsequent dehydration of the resulting alcohol **21** gave a mixture of the desired $\Delta^{7,8}$-olefin **22** and its $\Delta^{6,7}$-isomer (*ca.* 1 : 1) (49%, 2 steps). Subjection of this mixture with triflic acid at 95°C effected both cleavage of the MOM ether and isomerization of the $\Delta^{6,7}$-olefinic double bond, providing alcohol **23** as a sole product in 57% yield. Swern oxidation of **23** followed by sodium chlorite oxidation of the resulting aldehyde **24** furnished carboxylic acid **25**. Further transformation of **25** to **3**, mp 234-235 °C, was successfully achieved by employing the reaction sequence similar to that described for the preparation of **2** from **10**.

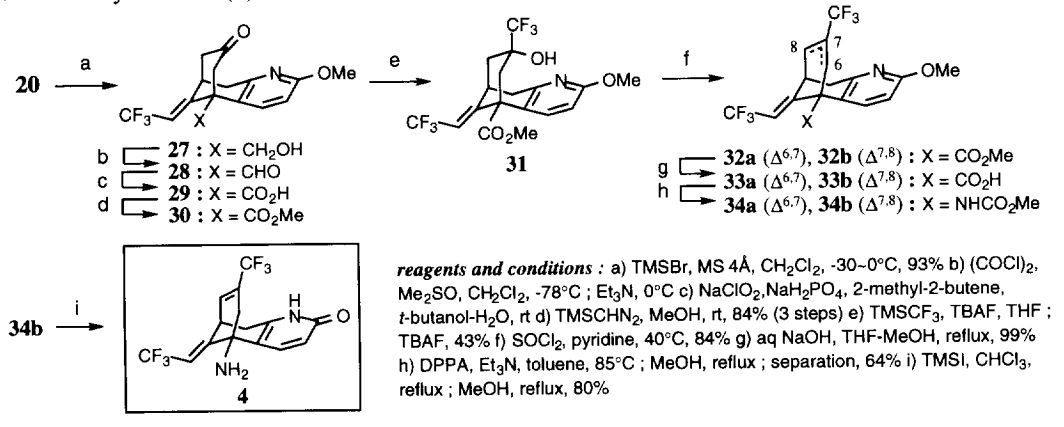
The final target compound, (±)-12,12,12,14,14,14-hexafluorohuperzine A (**4**), was synthesized starting from the key intermediate **20** as shown in **Scheme 3**. Thus, **20** was converted to methyl ester **30** through a four-step sequence of reactions involving cleavage of the MOM group, Swern oxidation of the resulting alcohol **27**, sodium chlorite oxidation of aldehyde **28**, and final esterification of the carboxylic acid **29** (78%, 4 steps).

Scheme 2. Synthesis of (±)-14,14,14-Trifluorohuperzine A (**3**)



reagents and conditions : a) CH_2=CHMgBr, THF, -78°C b) TMSOTf, 2,6-di-*t*-butylpyridine, CH_2Cl_2, rt, 51% (2 steps) c) DIBAL, CH_2Cl_2, -78°C, 68% d) MOMCl, *i*-Pr_2EtN, CH_2Cl_2, rt, 81% e) O_3, MeOH-CH_2Cl_2, -78°C ; Me_2S, rt, 71% f) $TMSCF_3$, TBAF, THF ; TBAF, 81% g) Im_2CS, toluene, reflux, 79% h) $P(OMe)_3$, 110°C, 92% i) MeLi, Et_2O-THF, -78°C, 51% j) $SOCl_2$, pyridine, 0°C, 96% k) TfOH, dioxane, 95°C, 57% l) $(COCl)_2$, Me_2SO, CH_2Cl_2, -78°C ; Et_3N, 0°C, 77% m) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-butanol-H_2O, rt, 99% n) DPPA, Et_3N, toluene, 85°C ; MeOH, reflux, 44% o) TMSI, $CHCl_3$, reflux ; MeOH, reflux, 74%

Scheme 3. Synthesis of (±)-12,12,12,14,14,14-Hexafluorohuperzine A (**4**)



reagents and conditions : a) TMSBr, MS 4Å, CH_2Cl_2, -30~0°C, 93% b) $(COCl)_2$, Me_2SO, CH_2Cl_2, -78°C ; Et_3N, 0°C c) $NaClO_2$,NaH_2PO_4, 2-methyl-2-butene, *t*-butanol-H_2O, rt d) $TMSCHN_2$, MeOH, rt, 84% (3 steps) e) $TMSCF_3$, TBAF, THF ; TBAF, 43% f) $SOCl_2$, pyridine, 40°C, 84% g) aq NaOH, THF-MeOH, reflux, 99% h) DPPA, Et_3N, toluene, 85°C ; MeOH, reflux ; separation, 64% i) TMSI, $CHCl_3$, reflux ; MeOH, reflux, 80%

Trifluoromethylation of **30** followed by dehydration of the resulting trifluoromethylated product **31** furnished an inseparable mixture of regioisomers **32a** and **32b** (*ca.* 1 : 3). Unfortunately, the undesired **32a** could not be transformed to **32b** by acid-catalyzed isomerization being different from the $\Delta^{6,7}$-isomer of **22**.[10] Therefore, the mixture of **32a** and **32b** was converted to the corresponding mixture of methyl carbamates **34a** and **34b** by way of carboxylic acids **33a** and **33b**. At this juncture, the regioisomers **34a** and **34b** could be readily separated by

Table 1. Inhibitory Activitiy Against Acetylcholinesterase (AChE)

Compound	IC_{50} (μM)
(–)-huperzine A (**1**)	0.005
(±)-12,12,12-trifluorohuperzine A (**2**)	0.4
(±)-14,14,14-trifluorohuperzine A (**3**)	2
(±)-12,12,12,14,14,14-hexafluorohuperzine A (**4**)	3

column chromatography on silica gel to produce the desired **34b** in 54% overall yield from **31**. Finally,methyl carbamate **34b** was similarly deprotected to furnish the requisite **4**, mp 249-250 °C, in 80% yield.

With the fluorinated analogues **2** - **4** in hand, *in vitro* AChE inhibitory activity of these compounds was assessed according to the method of Ellman *et al.*[11] Results shown in **Table 1** disclosed that all these analogues exhibit the activity inferior to that of (–)-huperzine A (**1**). Taking into account their racemic forms, analogue **2**, **3** and **4** were 40-, 200-, and 300-fold less potent than **1**, respectively. These findings suggest that the C-14 methyl group plays an important role in exhibiting significant AChE inhibitory activity. Therefore, it would be of interest to examine the activity of the analogues modified in the vicinity of the C-12 methyl group in **1**, and investigations in this direction are presently being pursued in our laboratories.

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1. Visiting scientist from Sankyo Co., Ltd.
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