An Improved Synthetic Route to Huperzine A; New Analogues and their Inhibition of Acetylcholinesterase

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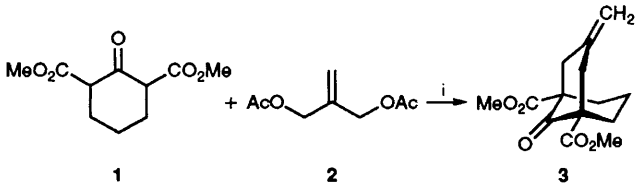
A bicycloannulation approach to huperzine A is detailed together with syntheses of the methylene analogue **8**, the diamino compound **12** and their inhibition of acetylcholinesterase.

As a consequence of the promise of huperzine A as a nootropic agent for use in the treatment of Alzheimer's disease,[1,2] we have been engaged in efforts to make this molecule and its analogues available for continued pharmacological and behavioural testing.[3,4] In order to expedite these aims, together with efforts to accelerate the clinical development of huperzine A, we have been investigating improved synthetic routes to the parent structure together with the synthesis and study of novel analogues. Herein we report an improved route to this molecule which makes huperzine A

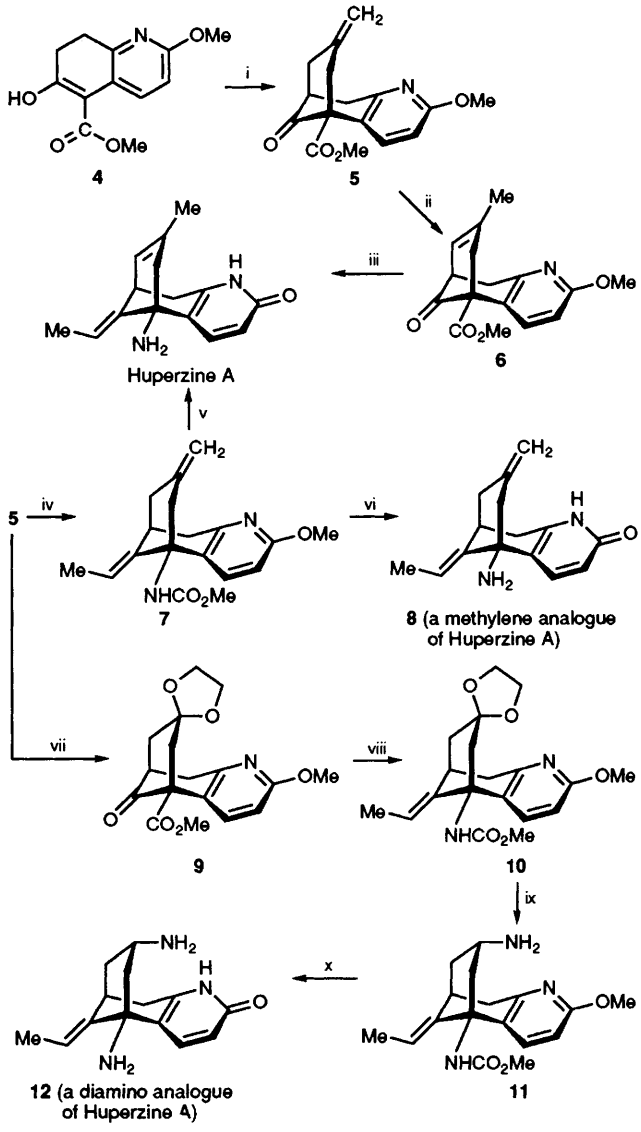


Scheme 1 *Reagents*: i, $Pd(OAc)_2$, ethylenebis(diphenylphosphine), *N*,*O*-bis(trimethylsilyl)acetamide

available in 40% overall yield from the previously reported β-keto ester starting material **4**. Additionally, we detail syntheses of both the methylene analogue **8** and the diamino compound **12** and their inhibitory activities for rat brain acetylcholinesterase. The preparation and study of compound **12** constituted one of the major reasons for undertaking this work, since this molecule was deemed to provide further information as to the correctness of the calculated binding site for the huperzine A molecule.[5]

The improved route to huperzine A is based upon work reported previously by Huang and Lu,[6] in which they showed that the bifunctional allylic alkylating agent **2** is capable of reacting with bifunctional nucleophiles, such as the keto diester **1**, under palladium catalysis to afford the bridged bicyclic product **3** in 58% yield (Scheme 1). Based upon this analogy, it was decided to try related chemistry on our fused ring methoxypyridine **4**.[4] We found that the diacetate of 2-methylenepropane-1,3-diol **2** could react with **4** in the presence of palladium diacetate, 1,1,3,3-tetramethylguanidine and triphenylphosphine to provide the methylene bridged structure **5** in 92% yield.[7] On exposing this compound to triflic acid in dioxane for 12 h, we obtained the isomerized product **6** in 90% yield. This compound was found to be identical to the material available from our previously reported route to huperzine A. The present chemistry thus makes it possible to bypass the mesylate elimination step employed in the earlier synthesis, the only poor yielding step in the entire reaction sequence.[3,4] It is also interesting to note that the double bond isomerization step can be carried out at the very last stage of the huperzine A synthesis. Thus, as shown in Scheme 2, transformation of the methylene intermediate **5** by the sequence of steps (iv) involving: Wittig reaction, *Z* to *E* isomerization of the exocyclic olefin and conversion of ester to urethane afforded structure **7**. When **7** was reacted with trimethylsilyl iodide (TMSI) in chloroform, partial isomerization of the exocyclic olefin was observed concomitant with the deprotection events. Triflic acid treatment as before then completed this second route to the natural product. By reacting **7** with lithium n-propanethiolate in hexamethylphosphoramide (HMPA), it was also possible to obtain the unrearranged amino pyridone, namely the isomeric hyperzine A analogue **8**, as shown in Scheme 1.†

To obtain the diamino compound **12**, the methylene intermediate **5** was treated with osmium tetroxide–sodium periodate to produce a diketone which could be selectively transformed to the monoethylene ketal **9**. Next, the ethylidene group was installed in the usual manner, the ester hydrolysed to acid and the Curtius rearrangement brought about to give urethane **10**. After ketal hydrolysis, reductive



Scheme 2 *Reagents and conditions*: i, 2-methylenepropane-1,3-diol diacetate, $Pd(OAc)_2$, PPh_3, 1,1,3,3-tetramethylguanidine, dioxane (92%); ii, TfOH, dioxane, 93 °C, resealable tube (90%); iii, same steps as reported in ref. 4; iv, a, $Ph_3P{=}CH{-}Me$, tetrahydrofuran (THF) (83%); b, PhSH, azoisobutyronitrile, PhMe, 85 °C (92%); c, 20% NaOH, MeOH, THF, reflux (83%); d, $(PhO)_2PON_3$, Et_3N, PhMe, reflux; MeOH, reflux (82%); v, a, TMSI, $CHCl_3$, reflux; MeOH, reflux; b, TfOH, dioxane, 93 °C, resealable tube (84% for the two steps); vi, Pr^nSLi, HMPA, 90 °C (87%); vii, a, OsO_4, 80% HOAc; $NaIO_4$, (89% overall); b, ethylene glycol, toluene-*p*-sulfonic acid (*p*-TSA), benzene, reflux (94%); viii, conditions of iv (88%, 94%, 81%, and 80% for the respective steps); ix, a 5% HCl, Pr^iOH, 70 °C (93%); b, NH_3, MeOH, NH_4Cl, 70 °C, resealable tube; $NaBH_4$, 0 °C (84%); x, TMSI, $CHCl_3$, reflux; MeOH, reflux (82%)

amination[8] to give **11**, and deprotection, the desired amine **12** was obtained. The stereochemistry of the amino group was assigned from NOESY and COSY experiments and is consistent with the approach of the hydride reagent from the less-hindered face.

While analogue **12** is now being used to study the effect of an extra charge on a ligand in relation to its kinetics of association with the cholinesterase enzyme,[9] we report here that **12** has an IC_{50} value of 8.1 ± 1.3 μmol dm^{-3} when tested for its ability to inhibit acetylcholinesterase from rat cortex.[10] The racemic diamino compound is thus about 110 times less active than racemic huperzine A. The methylene analogue **8** was also less potent than huperzine A, with an IC_{50} of 4.9 ± 1.5 μmol dm^{-3}.

† *Physical and spectral data* for **8**: colourless prisms from ethyl acetate; m.p. 295 °C (decomp.); R_F = 0.6 (15% MeOH in $CHCl_3$); IR ν/cm^{-1} (KBr) 3395–3480, 2911, 1660, 1610, 1558, 1462, 1111, 893; ^1H NMR (300 MHz, $CDCl_3$) δ 12.26 (br s, pyridone NH), 7.77 (d, 1H, *J* 9.4 Hz), 6.37 (d, 1H, *J* 9.4 Hz), 5.62 (q, 1H, *J* 6.7 Hz), 4.71 (m, 1H), 4.48 (m, 1H), 3.40 (m, 1H), 2.96 (dd, 1H, *J* 17.8, 6.9 Hz), 2.61 (m, 1H), 2.31–2.11 (m, 4H), 1.71 (d, 3H, *J* 6.7 Hz), 1.60 (br s, NH_2); ^{13}C NMR (75.46 MHz, CD_3OD) δ 13.5, 33.3, 36.4, 44.7, 53.9, 57.8, 114.3, 115.4, 118.5, 124.9, 141.7, 143.9, 145.7, 145.9, 165.4.

Since the palladium catalysed bicycloannulation route to huperzine A has been used to prepare multigram quantities of the compound, this route possesses certain advantages over our previously published route. Further details of the kinetic experiments with the new analogues will be reported separately.[11]

We are indebted to the National Institute on Aging for their support. We also thank Dr Y.-P. Pang for his assistance with the NMR experiments.

Received, 9th February 1993; Com. 3/00784G

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