JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 813–822.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.605

Research Article

Synthesis of a ^{13}C labeled *N*-cyclopropylamine tetrahydropyridine derivative

Simon Kuttab*[,1,2] and Stephan Mabic[2]
[1] *Department of Chemistry, Birzeit University, Birzeit, West Bank, Via Israel*
[2] *Department of Chemistry, Peters Center, Virginia Tech, Blacksburg, VA 24012–0212, USA*

Summary

The synthesis of 1-(2-^{13}C)-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine (8) is reported. Attempts were first made to prepare labeled cyclopropylamine via a cyclopropanation/Curtius rearrangement sequence, but the yields were too modest to be suitable for the synthesis of a labeled compound. The preparation of 8 was achieved via cyclopropanation of the *N*-formyl tetrahydropyridine derivative 21 using the Grignard reagent of ethyl bromide and Ti(O-*i*Pr)$_4$ as a catalyst. The synthesis proceeded in high yield (82%). The method has a wide potential for the synthesis of other cyclopropyl ring labeled and substituted cyclopropyl ring labeled tetrahydropyridine dervatives which can be used in Monoamine Oxidase (MAO) and Cyt P_{450} enzymes mechanistic studies.

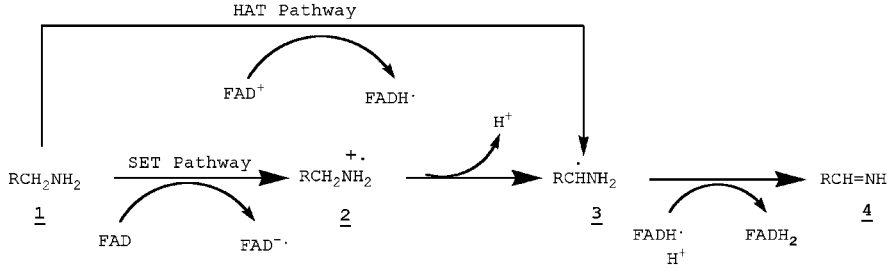
Key Words: *N*-formyl tetrahydropyridine; *N*-cyclopropyltetrahydropyridine; ^{13}C-label; 1-(2-^{13}C)-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine; monoamine oxidase

*Correspondence to: Dr. S. Kuttab, P.O. Box 19684, Jerusalem, via Israel 91196. E-mail: skuttab@birzeit.edu.
Contract/grant sponsor: National Institute of Neurological Disorders and stroke (NS 28792),
Contract/grant sponsor: Harvey W. Peters Research Center

Received 19 February 2002
Revised 22 March 2002
Accepted 4 April 2002

Introduction

In our ongoing efforts to investigate the enzymatic mechanism and inactivation of the flavin (FAD) containing enzymes monoamine oxidases A and B (MAO-A and MAO-B)[1–4], we have focused on the use of 1,4-disubstituted-1, 2, 3, 6-tetrahydropyridines as useful probes.[5–9] Two principal pathways have been proposed to account for the MAO-B catalytic pathway. The first involves an α-hydrogen atom transfer (HAT) in the initial step to directly generate 3 from 1 which subsequently goes to 4.[10–12] The alternative pathway involves an initial single electron transfer (SET) leading to the aminyl radical cation 2 followed by deprotonation and a second electron transfer to yield 4 (Scheme 1).[13–16]

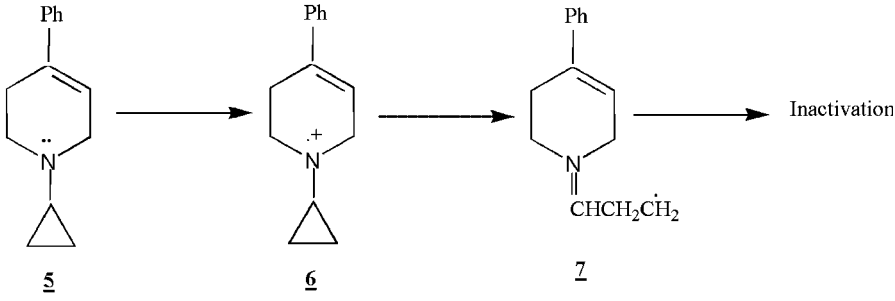


Scheme 1. SET and HAT pathways proposed for the MAO-B catalysed Oxidation of Amines

Studies with various cyclopropylamines have been offered as evidence in support of the SET pathway for the inactivation of both the Cytochrome P-450 family of enzymes[17–21] and the flavoenzymes Monoamine Oxidases (MAO) A and B.[22,23]

The inactivating properties of 1-cyclopropyl-4-phenyl-1,2,3,6 tetrahydropyridine 5 and other analogs[6,8,9,24] are consistent with the SET pathway. The inactivation may be considered to proceed via the cyclopropylaminyl radical cation 6 and the subsequent bioalkylation of the enzyme active site by the ring opened carbon centered radical 7 (Scheme 2), leading to loss of catalytic activity.

As part of our mechanistic studies, we have initiated efforts to synthesize various labeled cyclopropyl tetrahydropyridrine derivatives to enable us to characterize the inactivation pathway of MAO-B using various techniques and model reactions. To this aim, the synthesis of the ^{13}C labeled analog 8 was initially targeted with the labeling at the C_2

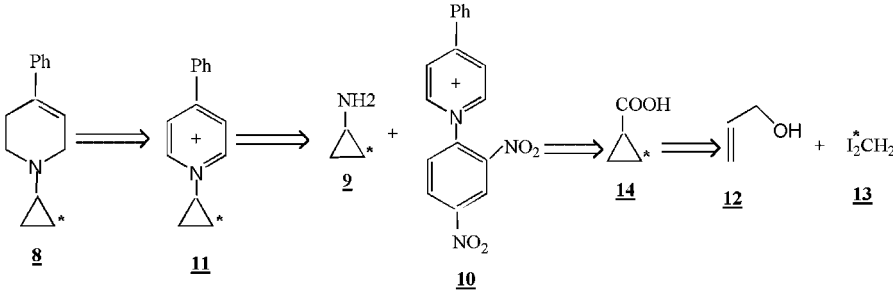


Scheme 2. SET pathway proposed for the inactivation of MAO-B by 5

carbon of the cyclopropyl ring in an effort to characterize the inactivation pathway of MAO-B by 8 using NMR spectroscopy.

Results and discussion

Our initial synthetic approach, described by the retrosynthetic path shown in Scheme 3, was to proceed via reaction of labeled cyclopropylamine 9 with the Zincke salt 10[16,24] to produce the *N*-cyclopropylpyridinium intermediate 11. Reduction of 11 with $NaBH_4$ in MeOH[25,26] yields 8.[16,27,28]

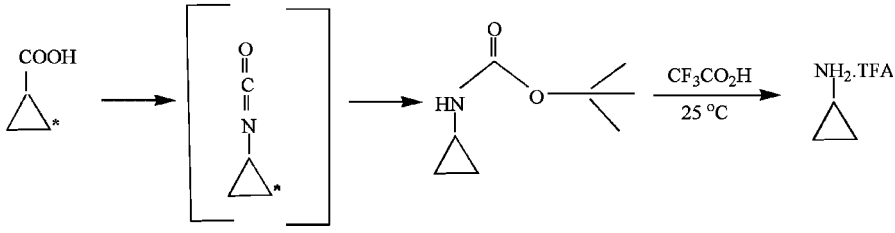


Scheme 3. Retrosynthesis to 8

A Simon–Smith type cyclopropanation of allylic alcohol 12 with labeled diiodomethane 13, and a Curtius rearrangement of the acid 14 were the key steps of the synthesis of labeled cyclopropylamine 9. The Curtius rearrangement first was studied in detail (Table 1). Because of the low boiling point (50°C) of 9, the *t*-Boc derivative 15 was prepared first (Scheme 4). The carbamate 15 was then efficiently (>98% yield) deprotected using trifluoroacetic acid (TFA), and cyclopropylamine was isolated as its non volatile TFA salt.[29]

Table 1 Curtius rearrangement of Cylopropanecarboxylic acid 14

Entry	Conditions	Yield (%)
1	DPPA, Net_3, *t*-BuOH, Δ, 5 h	73
2	DPPA, Net_3, *t*-BuOH, CuCl, Δ, 3h	5
3	DPPA, NET_3, *t*-BuOH, Toluene, Δ, 6 h	81
4	1- DPPA, NET_3, Toluene, Δ, 2-*t*-BuOH, Δ, 5 h	47
5	1-NEt_3, ClO_2Et, H_2O/Acetone, 0°C; 2-NaN_3; 3-*t*-BuOH, Δ, 14 h	22
6	1-$SOCl_2$, Δ, 1 h; 2-NaN_3, H_2O/Acetone, 0°C; 3-*t*-BuOH, Δ, 8 h	7

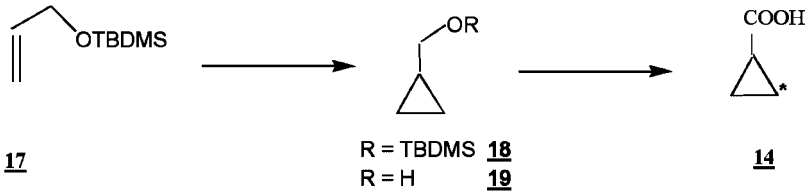


14 16 15 9

Scheme 4. Synthesis of Cyclopropylamine 9 from Cylopropane- carboxylic acid 14

The *in situ* formation of the isocyanate intermediate 16 using diphenylphosphoryl azide (DPPA), and reaction with *t*-BuOH gave the best yields (Table 1. Entries 1 and 3). 1,3-Dicyclopropylurea was obtained as a side product when the reaction was not performed under rigorously anhydrous conditions.[29,30] A slightly higher yield was obtained using toluene as a solvent (Entry 3). The higher temperature reached in toluene might favor the rearrangement. The use of CuCl (Entry 2) as well as running the two steps of the reaction sequentially (Entry 4)[31] results in a dramatic decrease in yield. Other methods (Entries 5[32,33], and 6[34]) gave much poorer yields presumably due to the low boiling point of the intermediates.

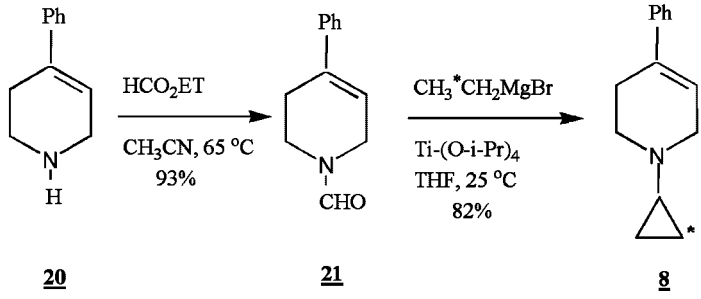
The preparation of cyclopropanecarboxylic acid 14 was examined next. Cyclopropanation of the TBDMS derivative 17 of allylic alcohol 12 is reported to proceed efficiently with $ClCH_2I$ to yield the silyloxymethylcyclopropane 18, (Scheme 5).[35,36] We examined the deprotection/oxidation sequence from 18 to the carboxylic acid 14. As expected, the silyl group could be easily removed using TBAF (91% yield). The intermediate alcohol 19 was oxidized to 14 with RuO_2 and $NaIO_4$ in 48% yield[32,35] and with $RuCl_3$ and $NaIO_4$ in 62% yield.[37] It occurred to us, however, that the conditions used for the oxidation



Scheme 5. Cyclopropanation of 17

should also allow for the deprotection of the silyloxy group to the alcohol. The compound 18 indeed was converted in one step and 78% yield to 14 using RuO_2 and $NaIO_4$. The efficient cyclopropanation reagent $ClCH_2I$ is not commercially available as a labeled compound, but $^{13}CH_2I_2$ can be purchased. Catalysis with $ZnEt_2$ was shown to be an efficient method to prepare a variety of cyclopropyl derivatives.[38–42] However, using one equivalent of CH_2I_2 only (instead of the usual 5–10 equivalents relative to the alkene) and an excess of alkene 17 led to modest yields of 18 and therefore this pathway was considered unsuitable for the synthesis of labeled 9.

An alternative synthetic approach was considered based on a recent literature report describing the synthesis of *N*-cyclopropyl dervatives from amides and Grignard reagents with a $Ti(O\text{-}i\text{-}Pr)_4$ catalyst.[43] The synthetic scheme (Scheme 6) is reduced to two steps from the commercially available tetrahydropyridine 20, and offers the advantage of being able to efficiently introduce the label in the last step.



Scheme 6. Synthesis of 8 from the Tetrahydropyridine 20

Various reagents were used to prepare the key intermediate 1-formyl-4-phenyl-1,2,3,6-tetrahydropyridine 21: ZrO_2, DMF (47% yield),[44] silicic acid, DMF (18%)[44] (The major product of this reaction was the 1-methyl-4-plenyl-1,2,3,6-tetrahydropyridine(52%)), carboxydiimidazole, formic acid (72% yield),[45] and ethyl formate in CH_3CN (93%

yield). The last method proved to be the simplest and the most efficient in our hands. Two ^{1}H NMR signals, in a ratio 2:1 are observed for the formyl hydrogen of 21. The major isomer exhibited a higher chemical shift (4.19 vs 4.07 for the minor rotamer) for the signals of hydrogen atoms at the C6 position, and a lower chemical shift (3.63 vs 3.79 for the minor rotamer) for the signals of the hydrogen atoms at the C2 position. This trend is consistent with an arrangement of the carbonyl group syn to the C6 position. The upfield chemical shifts are due to the deshielding effect of the carbonyl group.[46] The ^{13}C labeled 1-cyclopropyl-4-phenyl-1,2,3,6-tertrahydropyridine (8) was obtained in 82% yield by reaction of 21 with the ethyl Grignard reagent prepared from the labeled ethyl bromide, using $Ti(O\text{-}i\text{-}Pr)_4$ as a catalyst. The tetrahydropyridine 8 was isolated as its stable oxalate salt. The synthesis of 21 and its subsequent relatively facile conversion to the cyclopropyl ring substituted tetrahydropyridine dervative as described above broadens the range of synthetically accessible cyclopropyl ring labeled and substituted cyclopropyl ring labeled tetrahydropyridine derivatives at more than one position which can be used in Monoamine Oxidase (MAO) or Cyt P_{450} enzyme mechanistic studies. Efforts in this direction are currently underway.

Experimental

General procedures can be found in Reference 16. The spectroscopic data of the following compounds were consistent with literature reports: 8[8], 11[47], 15[48], 17[49], and 18[50]. The ^{13}C labeled ethyl bromide (99% enrichment) was purchased from Cambridge Isotope Laboratories.

Tert-butoxycarbonyl aminocyclopropane 15

The acid 14 (860 mg, 10 mmol) was dissolved in anhydrous *t*-BuOH and anhydrous toluene (20 ml). The NEt_3 (2 ml, 10.5 mmol) and diphenylphosphoryl azide (2.5 ml, 11.5 mmol) were added and the reaction mixture was heated up to reflux for 6 h. After cooling, the solvents were evaporated under reduced pressure and the crude residue was taken up in $CHCl_3$ (50 ml), washed with a solution of citric acid (10%, 40 ml), a solution of NaOH (2%, 25 ml), and water. After drying over $MgSO_4$ and evaporation of the solvents under reduced pressure, the crude product was purified by Al_2O_3 column chromatography ($CHCl_3$) to

give 15 as a white solid (1.25 g, 8.1 mmol, 81% yield), which was further purified by recrystallization from Hex/Et_2O at $-20^{\circ}C$. ^1H NMR ($CDCl_3$, 360 MHz) δ 4.7 (1H, bs) 2.53 (1H, m), 1.44 (9H, s), 0.67 (2H, m); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 79.4, 28.4, 22.9, 6.7; EIS m/z (rel intensities %) 157 (M^{+2}), 142(2), 101(17), 57(100), 41 (62). Anal. Calcd for $C_8H_{15}NO_2$; C, 61.12; H, 9.62; N, 8.91. Found: C, 61.21; H, 9.58; N, 8.97.

1-Formyl-4-phenyl-1,2,3,6-tetrahydropyridine 21

The freshly prepared free base 20 (2.54 g, 16 mmol) was dissolved in dry CH_3CN in a 50 ml round bottomed flask fitted with a reflux condenser. Ethyl formate (1.6 g, 21.8 mmol) was added and the reaction mixture was heated under nitrogen at 65–70°C for 48 h. The solvent was removed in vacuo and the thick, yellowish oily residue was dissolved in CH_2Cl_2 (30 ml). This solution was washed with dilute HCl (10%, 3×20 ml), dried over Na_2SO_4 and the solvent removed in vacuo to yield a yellowish solid, which was purified by SiO_2 column chromatography (CH_2Cl_2/MeOH 95:5) to give 21 as pale yellow crystals (2.6 g, 14 mmol, 88% yield), mp: 77–78°C. ^1H NMR ($CDCl_3$, 360 MHz, mixture of the two rotamers) δ 8.21 (1H, s), 8.15 (1H, s, ratio 1:2), 7.34 (5H, m), 6.05 (1H, m), 4.2 (2H, m), 4.06 (2H, m, ratio 2:1), 3.79 (2H, t, $j = 6.2$ Hz), 3.63 (2H, t, $j = 6.2$ Hz, ratio 1:2), 2.62 (2H, m), 2.57(2H, m, ratio 2:1); ^1H NMR (DMSO-d_6, 360 MHz, mixture of the two rotamers, ratio 1:2), δ 8.17 (1H, s), 8.12 (1H, s, ratio 1:2)), 7.30 (5H, m), 6.19 (1H, m), 6.14 (1H, m, ratio 1:2) 4.08 (2H, m), 4.04 (2H, m, ratio 1:2), 3.62 (2H, m) 2.52 (2H, m); ^{13}C NMR (DMSO-d_6, 90 MHz, mixture of the two rotamers)δ 161.4, 160.9, 139.8, 134.8, 134.6, 128.4, 127.6, 124.7, 120.6, 119.9, 44.2, 41.7, 36.1, 27.2, 25.9; GC-EIMS m/z (rel intensities %) 187 (M^+100), 158 (20), 143 (17), 130(24), 115(30), 91(26); UV (nm, MeOH) 208, 245. Anal. Calcd for $C_{12}H_{13}NO$: C, 76.96; H, 7.00; N, 7.48. Found: C, 76.89; H, 7.08; N, 7.55.

1-[2-^{13}C]-Cyclopropyl-4-phenyl-1,2,3, 6-tertrahydropyridine 8

Ethyl bromide 2-^{13}C (1.0 g, 9.17 mmol) was added slowly via a dropping funnel to magnesium turnings in dry THF under nitrogen. The reaction was heated under reflux for 3 h. Into a flame dried three-necked round bottomed flask fitted with a reflux condenser, a dropping funnel, a septum, and a magnetic stirrer, was added a solution of 21(0.68 g,

3.67 mmol) in dry THF. Titanium isopropoxide (1.14 g, 4.03 mmol) was added via the dropping funnel and the resulting ethyl magnesium bromide was immediately transferred via a syringe. The reaction mixture, which slowly turned black, was warmed (oil bath 60°C) and left to stir overnight (18 h). The reaction was cooled to 25°C and a saturated aqueous solution of ammonium chloride was added with stirring. A precipitate of titanium hydroxide formed. The reaction mixture was filtered through a sintered glass funnel and the filtrate extracted with ether (3×20 ml). The ether layer was washed with brine (2×20 ml), dried over Na_2SO_4 and evaporated to yield a yellowish solid. After column chromatography on Al_2O_3 (Hex/AcOEt 97:3) the desired product 8 was obtained as a white crystalline solid (82%): mp 63–64°C; ^{1}H NMR ($CDCl_3$) δ 7.30 (5H, m), 6.05 (1H, m), 3.31 (2H, m), 2.87 (2H, m), 2.55 (2H, bs), 1.73 (1H, m), 0.74 (1H, m), 0.49 (1H, m), 0.29 (1H, m); ^{13}C NMR ($CDCl_3$) δ 128, 126, 124, 122.53, 51.37, 29, 22, 20, 5.5; EIMS (m/z, rel int) 200 (71), 185 (100), 184 (76), 156 (15), 128(45), 115(61), 91(22), 69(36). The free base was then converted to the oxalate salt and recrystallized from MeOH/Et_2O(mp 175–176°C).

Acknowledgements

This work was supported by the National Institute of Neurological Disorders and Stroke (NS 28792) and the Harvey W. Peters Research Center.

References

1. Dosert P, Strolin-Benedetti M, Tipton KF. *Med Res Rev* 1989; **9**: 45–89.
2. Langston JW, Ballard P, Tetrud JW, Irwin I. *Science* 1983; **219**: 979.
3. Langston JW, Irwin I, Langston EB, Frono LS. *Neurosci Lett* 1984; **48**: 87.
4. Walker MC, Edmonson DE. *Biochemistry* 1994; **33**: 7088.
5. Kalkutgar AS, Castagnoli K, Hall A, Castagnoli Jr N. *J Med Chem* 1994; **37**: 944–951.
6. Kuttab S, Kalkutgar A, Castagnoli Jr N. *Chem Res Toxicol* 1994; **6**: 740–744.
7. Franot C, Mabic S, Castagnoli Jr N. *Bioorg Med Chem* 1998; **6**: 283–291.
8. Hall L, Murray S, Castagnoli K, Castognoli Jr N. *Chem Res Toxicol* 1992; **5**: 625–633.

9. Rimoldi JM, Wang YX, Nimkar SK, *et al. Chem Res Toxicol* 1995; **8**: 705–710.
10. Miller RJ, Edmonson DE, Grisson CB. *J Am Chem Soc* 1995; **117**: 7830–7831.
11. Silverman RB, Zelechonok Y. *J Org Chem* 1992; **35**: 6379–6384.
12. Silverman RB. In *Advances in Electron Transfer Chemistry*, vol. 2, Mariano PS (ed.). JAI Press: Greenwich, 1992; 177–213.
13. Silverman RB. *Accounts Chem Res* 1995; **28**: 335–342.
14. Silverman RB, Yamasaki RB. *Biochemistry* 1984; **23**: 1322–1331.
15. Silverman RB, Zhow JP, Eaton PE. *J Am Chem Soc* 1993; **115**: 8841–8842.
16. Nimkar SK, Anderson A, Rimoldi JM, *et al. Chem Res Toxicol* 1996; **9**: 1013–1022.
17. Bondon A, Macdonald TL, Harris TM, Guengerich FP. *J Biol Chem* 1989; **264**: 1988–1997.
18. Macdonald TL, Zirvi K, Burka LT, Peyman P, Guengerich FP. *J Am Chem Soc* 1982; **104**: 2050–2052.
19. Guengerich FP, Willard RJ, Shea JP, Richard LE, Macdonald TL. *J Am Chem Soc* 1984; **106**: 6446–6447.
20. Guengerich FP, Macdonald TL. In *Advances in Electron-Transfer Chemistry*, vol 3, Mariano PS (ed.), JAI Press: Greenwich, Connecticut, 1993; 191–241.
21. Hanzlik RP, Tullman RH. *J Am Chem Soc* 1982; **104**: 2048–2050.
22. Vasquez ML, Silverman RB. *Biochemistry* 1985; **24**: 6538–6543.
23. Mitchell DJ, Nikolic D, Rivera E, *et al. Biochemistry* 2001; **40**: 5447–5456.
24. Zincke T. *Chem Ber* 1904; **37**: 361–375.
25. Lyle RF, Nelson DA, Anderson PS. *Tetrahedron Lett* 1962; **4**: 553–556.
26. Anderson PS, Lyle RE. *Tetrahedron Lett* 1964; **6**: 153–156.
27. Wang XY, Castagnoli Jr N. *J Med Chem* 1995; **38**: 1904–1910.
28. Nimkar SK, Mabic S, Anderson AM, *et al. J Med Chem* 1999; **42**: 1828–1835.
29. Kimura Y, Atarashi S, Kawakami K, Sato K, Hayakawa I. *J Med Chem* 1994; **37**: 3344–3352.
30. Thornton TJ, Jarman MJ. *Synthesis* 1990; 295–299.
31. Vallgarda J, Appelberg U, Ardvisson IE, *et al. J Med Chem* 1996; **39**: 1485–1493.
32. Zhao Y, Yang T, Lee M, Lee D, Newton MG, Chu CK. *J Org Chem* 1995; **60**: 5236–5242.
33. Vangveravong S, Nichols DE. *J Org Chem* 1995; **60**: 3409–3413.
34. Rewcastle GW, Denny WA. *Synthesis* 1985; 220–223.
35. Zhao Y, Yang T, Lee M, *et al. Tetrahedron Lett* 1994; **35**: 5405–5408.
36. Denmark SE, Edwards JP. *J Org Chem* 1991; **56**: 6974–6981.
37. Wipf P, Xu W. *J Org Chem* 1996; **61**: 6556–6562.

38. Furukawa J, Kawabata N, Nishimura J. *Tetrahedron* 1968; **24**: 53–58.
39. Furukawa J, Kawabata N, Nishimura J. *Tetrahedron* 1968; **31**: 3495–3498.
40. Morikawa T, Sasaki M, Hanai R, Shibuya A, Taguchi T. *J Org Chem* 1994; **59**: 97–103.
41. Charette AB, Prescott S, Brochu CJ. *J Org Chem* 1995; **60**: 1081–1083.
42. Denmark SE, O'Connor SP. *J Org Chem* 1997; **62**: 584–594.
43. Chaplinski V, de Meijere A. *Angew Chem Int Ed Engl* 1996; **35**: 413–414.
44. Takahashi K, Slibagaki M, Matsushita H. *Agric Biol Chem* 1988; **52**: 853–854.
45. Katigawa T, Aria J, Nagahata A. *Chem Pharm Bull* 1994; **42**: 1655–1657.
46. Rubiralta M, Giralt E, Diez A. In *Piperidine: Studies in Organic Chemistry*, vol. 43, Elsevier: Amsterdam, Oxford, New York, Tokyo, 1991.
47. Genisson Y, Marazano C, Mehmandoust M, Gnecco D, Das BC. *Synlett* 1992; 431–434.
48. Bridges AJ. *J Heterocycl Chem* 1990; **27**: 1527–1536.
49. Ali AS, Khan JM, Wazeer MIM. *Tetrahedron* 1988; **44**: 5911–5920.
50. Nonhebel DC, Suckling CJ, Walton JC. *Tetrahedron Lett* 1982; **22**: 4477–4480.

