^{14}C-Labeling of a Tetrahydroacridine, a Novel CNS-Selective Cholinesterase Inhibitor

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SUMMARY

9-Amino-8-fluoro-2,4-methano-1,2,3,4-tetrahydroacridine citrate (SM-10888), a novel cholinesterase inhibitor, was labeled with carbon-14 at C9 of the tetrahydroacridine ring for use in metabolic studies. Carbonation of 2,6-difluorophenyllithium (3) with [^{14}C]carbon dioxide gave the acid (4). Chlorination of 4 followed by treatment of the resulting acid chloride with ammonia afforded the amide (5). Dehydration of 5 with thionyl chloride and subsequent displacement reaction with ammonia gave the aminobenzonitrile (7). Condensation of 7 with the ketone (8) in the presence of anhydrous zinc chloride yielded the aminoacridine (9), which was treated with citric acid to afford [9-^{14}C]SM-10888 (1). The overall yield of 1 was 37% from 2, and the specific activity was 1.35 GBq/mmol.

Key words: ^{14}C-labeling, cholinesterase inhibitor, tetrahydroacridine, aminobenzonitrile, cyclocondenstion

INTRODUCTION

Alzheimer's disease is a brain disorder of unknown etiology characterized by a progressive loss of memory and intellectual function. It was reported that a specific deficit in choline acetyltransferase was observed from patients with Alzheimer's disease.[1),2),3)] Moreover, various types of evidence have indicated that a selective loss of cholinergic function is involved in memory and learning in humans as well as animals.[4),5)] These findings encouraged clinical trials with cholinesterase inhibitors such as physostigmine.[6)] Summers et al. reported that

0362–4803/92/070553–08$09.00
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Received 4 March, 1992
Revised 16 March, 1992

oral administration of tacrine (9-amino-1,2,3,4-tetrahydroacridine), a potent cholinesterase inhibitor, significantly improved the symptoms of Alzheimer's disease.[7] Tacrine induced, however, peripheral cholinergic side effects such as excessive micturition, diarrhea and diaphoresis.[7] SM-10888, a tacrine derivative which has higher selectivity to the central nervous system (CNS) than tacrine, has attracted much attention as a cholinergic treatment in Alzheimer's disease.[8] In the course of further evaluation of this pharmaceutical, it was required to synthesize radioactive SM-10888. In this paper, we wish to report the synthesis of SM-10888 labeled with carbon-14 at C9 of the tetrahydroacridine ring.

RESULTS AND DISCUSSION

Several methods have been developed for the preparation of 9-amino-1,2,3,4-tetrahydroacridine.[9),10),11)] In order to apply these methods to the present work, we considered three different approaches shown in Fig. 1.

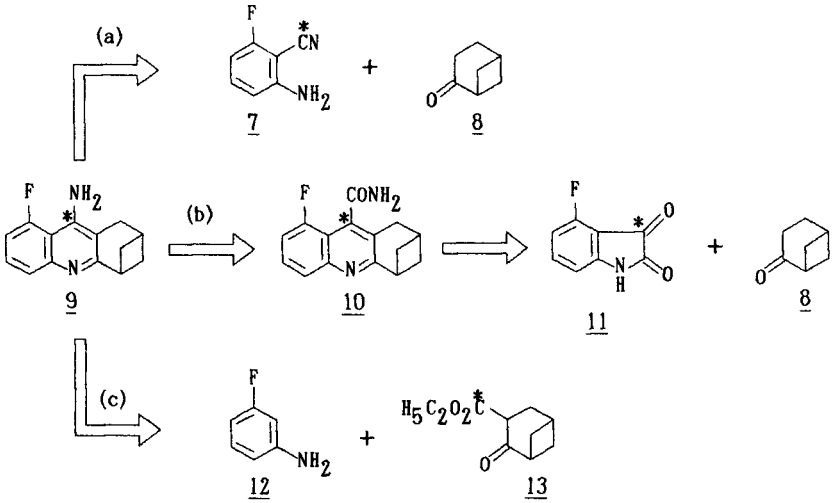

Fig. 1 Retrosynthetic Analysis of SM-10888

These methods include (a) cyclocondensation of aminobenzonitrile (7) with the ketone (8); (b) cyclocondensation of isatin (11) with 8 and aqueous concentrated ammonia followed by Hoffman degradation; (c) cyclocondensation of aniline (12) with the keto ester (13). In contrast to the

synthesis of tacrine, it was predicted that the approach (c) gave a serious problem that cyclocondensation of fluoroaniline (12) with the ketone (13) would afford two regioisomers. The approach (b) was considered impractical because of the tedious process and predicted lower yield in multistep synthesis of the isatin derivative (11). We focused, therefore, on the approach (a) for construction of the tetrahydroacridine skelton.

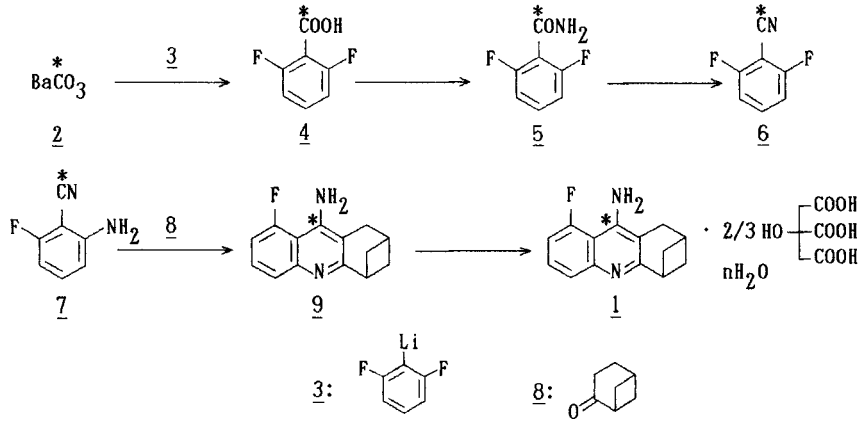

Fig. 2 Synthetic Procedure of [9-^{14}C]SM-10888

Fig. 2 illustrates the synthetic procedure of [9-^{14}C]SM-10888 (1). The key issue in the preparation of 2-amino-6-fluorobenzonitrile (7) was the introduction of three different substituents on adjacent carbons of the benzene ring. We paid attention to the fact that fluorine atom of 2,6-difluorobenzonitrile (6) was readily subjected to substitution to give the desired aminobenzonitrile (7). Consequently, synthesis of 6 was the major task for the present labeling work. Initial attempts to replace the amino group of 2,6-difluoroanilin with the cyano group through diazonium salt afforded 6 in poor yield. Other possibilities were, therefore, explored. Roe et al. reported the preparation of 2,6-difluorophenyllithium from 1,3-difluorobenzene and its conversion to a range of 2,6-difluoroaromatic compounds.[12] Application of this method to the radiosynthesis was achieved as follows. The lithio derivative (3) prepared by treating 1,3-difluorobenzene with n-butyllithium was allowed to react with [^{14}C]carbon dioxide to give 4 in 99% yield. The acid (4) was converted to the

corresponding acid chloride and then treated with ammonia water to yield 5 in 90% yield. Dehydration of the amide (5) with thionyl chloride gave 6 in 93% yield.

With the key intermediate in hand, we proceeded to the displacement reaction of 6. Thus, the benzonitrile (6) was reacted with ammonia in a pressure vessel to afford 7 in 78% yield. After purification, the aminobenzonitrile (7) was allowed to react with the ketone (8) in the presence of anhydrous zinc chloride to give 9 in 69% yield. The resulting tetrahydroacridine (9) was treated with citric acid to afford 1 in 87% yield. The overall yield of 1 was 37% from 2, and the specific activity was 1.35 GBq/mmol.

EXPERIMENTAL

Radioactivity was measured by a TRI-CARB 460 liquid scintilation counter (Packard Instrument Co., USA) by using Permafluor I (Packard) as the counting medium. Radio-thin layer chromatography (RTLC) was carried out on a Silica Gel 60 F_{254} (Merck), and the radioactivity on the plate was determined by a JTC-601 Radiochromanyzer (Aloka, Japan). Radio-high performance liquid chromatography (RHPLC) was conducted on a LC-3A high performance liquid chromatograph (Shimadzu Co., Ltd., Japan) equipped with a SPD-2A UV detector (Shimadzu Co.) and RLC-551 Radioanalyzer (Aloka). A stainless steel column packed with octadesyl silane (SUMIPAX ODS A-212, 5 μm, 6 mmi.d. x 15 cm, Sumica Chemical Analysis Service Ltd., Japan) was used for the analysis of 1 and 9. Operating condition: mobile phase 0.01M H_3PO_4 (0.2% triethylamine pH 2.5)/acetonitrile=17/3 v/v; flow rate 1.0 ml/min; detector UV (254 nm); retention time 13.6 min. An infrared spectrum (IR) was measured by a IR-810 grating infrared spectrophotometer (Jasco Co., Ltd., Japan), and the characteristic absorptions (ν_{max}) were reported in cm^{-1}. A proton nuclear magnetic resonance spectrum (NMR) was determined on a JNM FX-100 spectrometer (JEOL Ltd., Japan), and the chemical shifts

(δ) for protons were quoted in ppm downfield from tetramethylsilane as the internal standard. A mass spectrum was obtained on a Hitachi DF/GC/MS M-80B and DPS M-0101 (3 kV) spectrometer (Hitachi Ltd., Japan).

2,6-difluoro[carboxyl-^{14}C]benzoic acid (4)

Under a nitrogen atmosphere, to a solution of 1,3-difluorobenzene (2.92 ml) in anhydrous tetrahydrofuran (50 ml) was added a solution of n-butyllithium (1.6M in hexane solution) (15.4 ml, 24.6 mmol) at -78 °C, and the mixture was stirred at the same temperature for 3.5 h. To this solution was introduced at -78 °C [^{14}C]carbon dioxide liberated from barium [^{14}C]carbonate (2) (1.10 GBq, 1.00 g, 5.06 mmol), and the mixture was stirred at the same temperature for 1 h. After addition of 5% aqueous sodium hydoxide, the mixture was further extracted with 5% aqueous sodium hydoxide. The aqueous phase was washed with ether and acidified with concentrated hydrochloric acid. The aqueous phase was extracted with chloroform and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 4 (1.09 GBq, 99.0%). The purity 98.5% on RTLC (chloroform/methanol/acetic acid=40/2/1 v/v/v, Rf=0.20). NMR (δ, ppm, DMSO-d_6): 7.08-7.31 (2H, m, aromatic), 7.45-7.67 (1H, m, aromatic). IR (ν_{max}, cm^{-1}, nujol): 1700 (C=O).

2,6-difluoro[carbonyl-^{14}C]benzamide (5)

A mixture of the acid (4) (1.09 GBq, 797 mg, 5.04 mmol) and thionyl chloride (1.6 ml, 22.0 mmol) in anhydrous benzene (6.0 ml) was refluxed for 2 h. After evaporation of the solvent and thionyl chloride, the residue was cooled to 0 °C. To this residue was added ammonia water (28%) (6.0 ml), and the mixture was stirred at 0 °C for 1 h. After addition of water, the mixture was extracted with ethyl acetate. The organic phase was washed with water and saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 6 (977 MBq, 89.5%). The purity 98.8% on RTLC (cnloroform/methanol=20/1 v/v, Rf=0.23).

NMR (δ, ppm, DMSO-d_6): 7.01-7.54 (3H, m, aromatic), 7.81 (1H, s, amido), 8.10 (1H, s, amido). IR (ν_{max}, cm^{-1}, nujol): 1650 (C=O).

2,6-difluoro[cyano-^{14}C]benzonitrile (6)

To a solution of the amide (5) (977 MBq, 702 mg, 4.47 mmol) in anhydrous N,N-dimethylformamide (4.0 ml) was added a solution of thionyl chloride (0.70 ml, 9.64 mmol) in anhydrous N,N-dimethylformamide (3.0 ml), and the mixture was heated at 85 °C for 30 min. After addition of water, the mixture was extracted with ether. The organic phase was washed with 5% aqueous sodium carbonate solution, water and saturated sodium chloride solution successively and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed on silica gel with dichloromethane/hexane (1/1 v/v) to afford 6 (903 MBq, 92.8%). The purity 98.4% on RTLC (dichloromethane/hexane=1/1 v/v, Rf=0.31). NMR (δ, ppm, $CDCl_3$): 6.95-7.54 (2H, m, aromatic), 7.46-7.78 (1H, m, aromatic). IR (ν_{max}, cm^{-1}, liquid film): 2240 (CN).

3-Amino-6-fluoro[cyano-^{14}C]benzonitrile (7)

A solution of the benzonitrile (6) (903 MBq, 927 mg, 6.67 mmol) in ethanol saturated with ammonia (15.0 ml) was heated at 135 °C in a pressure vessel. The mixture was poured into water, and the resulting precipitate was filtered. The crystalline product was washed with water, toluene/hexane (1/1 v/v) and water successively. Traces of water was removed by azeotropic distillation with benzene and the remaining solid was dried under reduced pressure to give 7 (699 MBq, 77.5%). The purity 99% on RTLC (chloroform/methanol=20/1 v/v, Rf=0.43). NMR (δ, ppm, $CDCl_3$): 4.54 (2H, s, amino), 6.36-6.54 (2H, m, aromatic), 7.13-7.40 (1H, m, aromatic). IR (ν_{max}, cm^{-1}, nujol): 3450 3360 (NH_2), 2225 (CN).

9-Amino-8-fluoro-2,4-methano-1,2,3,4-tetrahydro[9-^{14}C]acridine (9)

Under a nitrogen atmosphere, a mixture of the aminobenzonitirile (7) (699 MBq, 770 mg, 5.61 mmol) and anhydrous zinc chloride (1.91 g, 14.0 mmol) in nitrobenzene (13.0 ml) was heated at 105 ℃. To this solution was added dropwise the ketone (8) (740 mg, 6.72 mmol) in nitrobenzene (2.0 ml), and the mixture was stirred at the same temperature for 6 h. After cooling to room temperature, 10% ammonia water (30.0 ml) was added, and the mixture was extracted with toluene. The organic phase was extracted with 5% hydrochloric acid, and the aqueous phase was washed with toluene. After neutralization with 28% ammonia water and subsequent extraction with toluene, the organic phase was dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was recrystallized from toluene (6.0 ml) and dried under reduced pressure to afford 9 (480 MBq, 68.8%). The purity 99% on RTLC (hexane/ethyl acetate/methanol/28% ammonia water=250/250/10/1 v/v/v/v, Rf=0.12). NMR (δ, ppm, $CDCl_3$): 1.55-1.66 (2H, m, C3), 2.41-2.65 (2H, m, methano), 2.76 (2H, d, J=2.8 Hz, C1), 2.80-3.00 (1H, m, C2), 3.24-3.43 (1H, m, C4), 5.14 (2H, s, amino), 6.82-7.09 (1H, m, aromatic), 7.26-7.72 (2H, m, aromatic). IR (ν_{max}, cm^{-1}, nujol): 3500 3400 (NH_2). MS (FD, m/z): 228, 230 (M^+).

9-Amino-8-fluoro-2,4-methano-1,2,3,4-tetrahydro[9-^{14}C]acridine citrate (SM-10888) (1)

Citric acid monohydrate (482 mg, 2.29 mmol) was dissolved in water (4.85 ml) at room temperature. To this solution was added the aminoacridine (9) (480 MBq, 810 mg, 3.55 mmol), and the mixture was heated at 85 ℃. After cooling to 70 ℃, the mixture was stirred at the same temperature for 2 h. Over a period of 2 h, the mixture was cooled to room temperature and then furthur cooled to 0 ℃ and stirred at the same temperature for 1 h. The crystalline product was filtered, washed with water and dried under reduced pressure to give 1 (418 MBq, 86.9%). The purity

99% on RTLC (hexane/ethyl acetate/methanol/28% ammonia water=100/100/30/1 v/v/v/v, Rf=0.50; chloroform/methanol/triethylamine=40/4/1 v/v/v, Rf=0.51) and RHPLC.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Y. Ouchi for unlabeled authentic samples and helpful discussions.

REFERENCES

1) Davies, P. and Maloney, A.J.F. - Lancet 2: 1403 (1976)

2) Bowen, D.M., Smith, C.B., White, P. and Dawson, A.N. - Brain 99: 459-496 (1976)

3) Perry, E.K., Perry, R.H., Blessed, G. and Tomolison, B.E. - Lancet 1: 189 (1977)

4) Drachman, D.A. and Lavitt, J. - Arch. Neurol. 30: 113-121 (1974)

5) Drachman, D.A. - Neurology 27: 783-790 (1977)

6) Thal, L.T., Fuld, P.A. Mathur, D.M. and Sharpless, N.S. - Ann. Neurol. 13: 491-496 (1983)

7) Summers, W.K., Majorsky, L.V., Marsh, G.M., Tachiki, K. and Kling, A. - N. Engl. J. Med. 315: 1241 (1986)

8) Kawasaki, H., Ohuchi, R., Kitano, M. and Ono, K. - (Sumitomo Pharm. Co., Ltd.) EP 268871, JP 88225358, JP 88239271, JP 89000073

9) Moore, J.A. and Kornreich, L.D. - Tetrahedron Lett. 20: 1227-1281 (1963)

10) Bielavsky, J. - Collection Czechoslov. Chem. Commun. 42: 2802-2808 (1977)

11) Petrow, V. - J. Chem. Soc. 634-637 (1947)

12) Roe, A.M., Burton, R.A., Willey, G.L., Baines, M.W. and Rasmussen, A.C. - J. Med. Chem. 11: 814-819 (1968)