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Research Article

Synthesis of *N*-(2-chloro-3,4-dimethoxybenzylideneamino)guanidinium acetate [α-^{14}C]

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Summary

^{14}C-Labelled *N*-(2-chloro-3,4-dimethoxybenzylideneamino)guanidinium acetate has been synthesized as a part of a four-step procedure which involved decarboxylation of 2-chloro-3,4-dimethoxybenzoic acid by $Pb(OAc)_4$ to give 2-chloro-3,4-dimethoxy-1-iodobenzene, followed by a selective lithiation at the iodine position and electrophilic substitution with *N,N*-dimethylformamide [α-^{14}C] and final reaction with aminoguanidine bicarbonate. The specific activity was 59 mCi/mmol and the overall yield 49%. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: *N*-(2-chloro-3,4-dimethoxybenzylideneamino)guanidinium acetate; 2-chloro-3,4-dimethoxy-1-iodobenzene; oxidative decarboxylation

Introduction

The compound *N*-(2-chloro-3,4-dimethoxybenzylideneamino)guanidinium acetate (**1**) has been shown to have a protective effect on tissue following ischemia reperfusion injuries in several animal models. The effect is partly due to an inhibitory effect on xanthine oxidase followed

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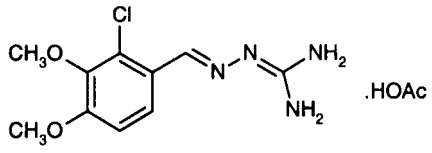

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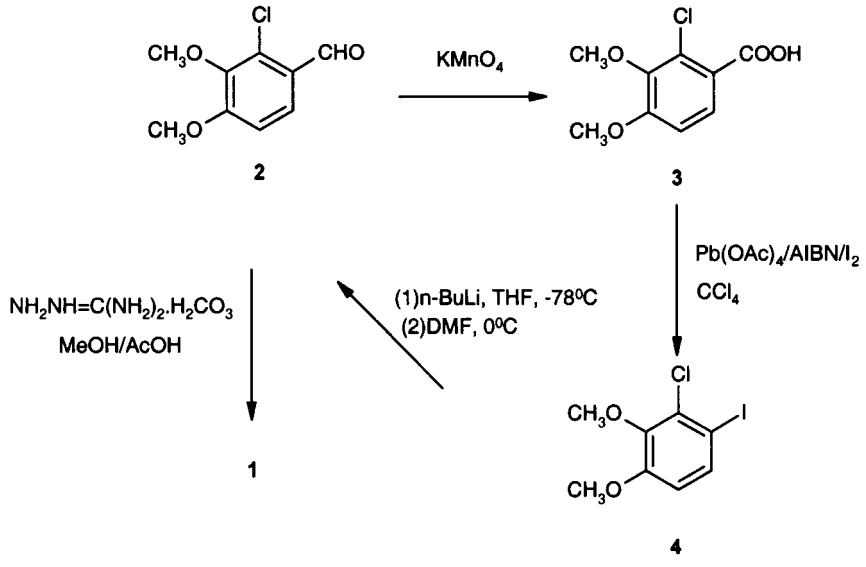
by a decrease in the concentration of oxygen-free radicals, which results in a tissue protective effect. Compound **1** decreased the mortality and arrhythmia in a dose-dependent manner rat model after occlusion of one cardiac artery.[1] In further pharmacological evaluation such as administration distribution metabolism elimination (ADME) and metabolic studies, it became clear that a radioactive form of **1** was required. The present work describes the methodology developed for the synthesis of *N*-(2-chloro-3,4-dimethoxybenzylideneamino)guanidinium acetate [α-^{14}C].

Results and discussion

The synthesis of non-radioactive *N*-(2-chloro-3,4-dimethoxybenzylideneamino)-guanidinium acetate (**1**) was performed in good yield (75%) by the reaction of aminoguanidinium bicarbonate with commercially available 2-chloro-3,4-dimethoxy-benzaldehyde (**2**).[2] This condensation reaction would be the last step in the synthesis of the desired labelled substrate. However, the synthesis of the [carbonyl-^{14}C]-**2** was not straightforward and presented difficulties. The initial idea was to start from the aldehyde **2**, remove the functional group and then restore the aldehyde function using a ^{14}C-labelled reagent. Several possibilities were tried for the removal of the carbonyl group (decarbonylation of aldehydes,[3] acyl halides[4] and oxidative decarboxylation of carboxylic acids[5]) and for the subsequent acylation step (Grignard and organolithium reagents[6]).

The decarbonylation of the aldehyde **2** to 3-chloro-1,2-dimethoxybenzene (**5**), could be accomplished in a moderate yield (45%) using a rhodium complex ($(PPh_3)_3RhCl$) in refluxing toluene or acetonitrile.[3] The successful ortho-lithiation of chlorobenzenes using *sec*-BuLi in THF at –105°C has been reported.[7c] This prompted us to try the same reaction conditions for compound **5**. Unfortunately, the lithiation was extremely sluggish and only the starting material could be detected by ^{1}H NMR.



Scheme 1.

The corresponding acyl halide, 2-chloro-3,4-dimethoxybenzoyl-bromide (**6**), was easily prepared by radical-promoted oxidation of the aldehyde with *N*-bromo-succinimide (NBS) in the presence of catalytic amounts of 2,2′-azobisisobutyronitrile (AIBN) as radical initiator.[8] Again, the attempted decarbonylation of compound **6** using $(PPh_3)_3RhCl/I_2$ in refluxing toluene[4] to afford compound **4** failed.

However, an alternative approach (Scheme 1) involving the oxidative decarboxylation of the corresponding carboxylic acid **3** was successful. The aldehyde **2** was readily oxidized using classical methods[9] giving the acid **3** in 76% yield and high purity, while the acid could be used directly in the next key step. Thus, the decarboxylation was accomplished in an easy and clean manner using $Pb(OAc)_4/I$[5d] and catalytic amounts of AIBN in CCl_4 to afford 2-chloro-3,4-dimethoxy-1-iodobenzene (**4**) in good yield (72%) after column chromatography. Having the key intermediate **4**, the acylation at the iodine position could, in principle, be performed either by forming the Grignard or the organolithium reagent.[6] The Grignard reaction was found to be sluggish and a rather complex mixture was obtained. On the other hand, the lithiation of **4** by metal–iodine exchange followed by the introduction of the formyl group using *N,N*-dimethyl-formamide in THF worked well and the aldehyde **2** was obtained in 65% yield after purification. It is worth mentioning that the acylation of the lithiated compound was sensitive to the acylating

reagent. When *N*-formyl-morpholine was used, no desired aldehyde was obtained and the ^{1}H NMR spectrum of the crude mixture showed 3-chloro-1,2-dimethoxybenzene as the main product.

The last step in the synthetic procedure involved the condensation of the aldehyde **2** with aminoguanidine to afford **1** in 75% yield. With the acquisition of the labelled aldehyde by using DMF [α-^{14}C] (59.6 mCi/mmol), radioactive **1** could be easily obtained in 99.5% radiochemical purity.

Conclusion

We have developed a simple method for the labelling of the title compound. Sisenwine *et al.*[10] have reported the synthesis of *N*-(2,6-dichlorobenzylidene-amino)guanidinium acetate [α-^{14}C]. They used a three-step synthesis in which the labelling of the desired carbon is accomplished in the first step but only with an overall yield of 12%. In our approach, the labelling of the aldehyde is performed one step before the final reaction and, moreover in 49% overall yield.

Experimental

Melting points were recorded on an Electrothermal IA9200 apparatus and are uncorrected. All solvents applied as reaction media were of analytical grade and dried for several days over molecular sieves (4 Å). Major chemicals were purchased from Maybridge and Aldrich and used as received. TLC analyses were performed on Merck silica gel (60 F_{254}) plates (0.25 mm), precoated with a fluorescent indicator. Visualization was effected with UV light, I_2 atmosphere, or phosphomolybdic acid reagent (PMA) 10% solution in ethanol. Column chromatography was carried out on Aldrich silica gel 60 (70–230 mesh). NMR spectra were routinely recorded in $CDCl_3$ on a Bruker Avance-300 instrument at 300 MHz (^{1}H) and 75 MHz (^{13}C). Chemical shifts are measured in parts per million (ppm) relative to chloroform (7.25 and 77.0 ppm) as internal reference. GC-MS analyses were performed on a Thermo-Quest Italia S.p.A. GC 8000 Top Series gas chromatograph coupled to a Finnigan Voyager mass spectrometer using a HP-5MS column (60 m $\times$ 0.25 mm $\times$ 0.25 μm) and helium as a carrier gas at an inlet pressure of 160 kPa. The samples were injected in a split mode at a ratio of 50:1 with an injector temperature at 220°C and column oven temperature at 280°C. The mass spectra were recorded at an electron energy of 70 eV and the ion source temperature was at 200°C.

2-chloro-3,4-dimethoxybenzoic acid (3)

A solution of potassium permanganate (3.40 g, 21.0 mmol) in water (150 ml) was added dropwise to a stirred slurry of compound **2** (3.00 g, 15.0 mmol) in water (100 ml) at 75°C. After addition (15–20 min), stirring was continued for a further 2 h at 75°C. The brownish reaction mixture was made alkaline (10% NaOH solution) and the mixture was filtered while hot. The manganese dioxide was washed with warm water (3×100 ml). The combined filtrate and washings were once again cooled and filtered to remove any starting material. The filtrate was acidified with HCl until no further precipitate formed. The resulting white precipitate was filtered, washed with cold water and dried to afford 2.48 g (76%) of the product as a white solid identical with an authentic sample (commercially available). The product was used without further purification.

2-chloro-3,4-dimethoxy-1-iodobenzene (4)

The acid **3** (2.97 g, 13.7 mmol), lead tetraacetate (6.70 g, 15.0 mmol), iodine (3.50 g, 15.0 mmol) and AIBN (66 mg, 0.40 mmol) were weighed into a two-necked flask connected to a condenser under N_2. Dry, deoxygenated CCl_4 (120 ml) was added via cannula and the resulting reaction mixture refluxed with stirring for about 2 h. The reaction mixture was cooled, diluted with CH_2Cl_2 (100 ml) and filtered. The organic phase was then washed with 1 M Na_2SO_3 (4×50 ml), saturated NaCl solution (4×50 ml), dried (anhydrous $MgSO_4$), and evaporated to afford 3.49 g of the crude product as a yellowish solid. Purification by column chromatography (silica gel, *n*-hexane/diethyl ether 2:1) gave 2.96 g (72%) of the pure product as white crystals: mp 69.8–70.2°C; ^{1}H NMR δ 7.56 (d, 1 H, $J = 8.8$ Hz), 6.64 (d, 1 H, $J = 8.8$ Hz), 3.88 (s, 3 H), 3.97 (s, 3 H); ^{13}C NMR δ 154.5, 146.5, 134.7, 133.5, 113.0, 87.7, 61.1, 56.6; MS *m*/*z* (relative intensity) 298 (M^+, 100).

2-chloro-3,4-dimethoxybenzaldehyde (2)

n-Butyllithium (500 µl of a 2.5 M solution in hexane, 1.25 mmol) was added dropwise via syringe under nitrogen to a solution of 2-chloro-3,4-dimethoxy-1-iodobenzene (**4**) (300 mg, 1.00 mmol) in dry THF (25 ml) cooled to –78°C. The mixture was stirred for 2 h at –78°C. Dry DMF (116 µl, 1.5 mmol) was added dropwise via syringe. The mixture was

then brought to 0°C and stirred for 1 h. The reaction was quenched with dilute HCl (1 M) until the solution became acidic. The mixture was extracted with diethyl ether (100 ml) and the organic layer washed in turn with water (3×25 ml), 1 M $NaHCO_3$ (3×25 ml), saturated NaCl solution (3×25 ml), dried (anhydrous $MgSO_4$), and finally the solvent was evaporated. The crude product was then purified by silica gel (hexane/diethyl ether 3:1) to afford 130 mg (65%) of the product as a white solid identical with an authentic sample (commercially available).

N-(2-chloro-3,4-dimethoxybenzylideneamino)guanidinium acetate (1)

Amino-guanidinium bicarbonate (150 mg, 1.10 mmol) was suspended in methanol (25 ml). The mixture was heated to 55°C and acetic acid (25 ml) was added. To the resulting clear solution, 2-chloro-3,4-dimethoxybenzaldehyde (201 mg,1 mmol) was added in one portion. The reaction mixture was refluxed until no aldehyde remained (<1% aldehyde (TLC), approximately 1 h). The methanol was removed and the residue was crystallized from ethanol. Purification of the crude product by HPLC (Lichrocart Select B (250×4.0 mm), 1.3 mM ammonium acetate + 0.08% formic acid: acetonitrile (1:1), isocratic, flowrate: 0.7 ml/min) afforded the product in 75% yield as a white powder: mp 200°C; ^{1}H NMR δ 8.32 (s, 1 H), 7.95 (d, 1 H, $J = 9$ Hz), 7.07 (d, 1 H, $J = 9$ Hz), 3.87 (s, 3 H), 3.75 (s, 3 H), 1.80 (s, 3 H); ^{13}C NMR δ 176.1, 158.0, 154.6, 144.8, 140.9, 127.4, 125.5, 122.8, 112.1, 60.5, 56.5, 24.0. Radioactive **1** was obtained in 99.4% radiochemical purity as indicated by HPLC (conditions as above) with a specific activity of 59 mCi/mmol as determined by MS.

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References

1. Dambrova M, Veveris M, Cirule H, *et al*. *Eur J Pharm*, accepted for publication.
2. Dambrova M, Piskunova J, Rozhkov E, *et al*. Patent W00125192, 2001.

3. Ohno K, Tsuji J. *J Am Chem Soc* 1968; **90**: 99–107.
4. Blum J, Rosenman H, Bergmann ED. *J Org Chem* 1968; **33**: 1928–1930.
5. (a) Sheldon RA, Kochi JK. *Org React* 1972; **19**: 279–421. (b) Barton DHR, Faro HP, Serebryakov EP, Woolsey NF. *J Chem Soc* 1965; 2438–2444. (c) Laszlo SE, Williard P. *J Am Chem Soc* 1985; **107**: 199–203. (d) Della EW, Head NJ. *J Org Chem* 1992; **57**: 2850–2855. (e) Ötvös L, Béres J, Sági G, Tömöskövi I, Gruber L. *Tetrahedron Lett* 1987; **28**: 6381–6384. (f) Conceptión JI, Francisco CG, Freire R, Hernández R, Salazar JA, Suárez E. *J Org Chem* 1986; **51**: 402–404.
6. (a) Olah GA, Ohannesian L, Arvanaghi M. *Chem Rev* 1987; **87**: 671–686. (b) Olah GA, Prakash KL, Arvanaghi M. Synthesis 1984; 228–230. (c) Smith JG, Dibble PW, Sandborn RE. J *Org Chem* 1986; **51**: 3762–3768.
7. (a) Silva SO, Reed JN, Snieckus V. *Tetrahedron Lett* 1978; 5099–5102. (b) Beak P, Snieckus V. *Acc Chem Res* 1982; **15**: 306–312. (c) Iwao M. *J Org Chem* 1990; **55**: 3622–3627.
8. Markó IE, Mekhalfia A. *Tetrahedron Lett* 1990; **31**: 7237–7240.
9. Shriner, RL, Kleiderer EC. *Org Syn* 1943; **2**: 538–539.
10. Sisenwine SF, Tio CO, Ahern J. *J Label Compd Radiopharm* 1976; **12**: 501–505.

