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Research Article

^{13}C- and ^{14}C-labelling of *N*-[1-(4-chlorophenyl)-1H-pyrrol-2-yl-methyleneamino] guanidinium acetate

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Summary

N-[1-(4-chlorophenyl)-1H-pyrrol-2-yl-$^{13}C_4$-methyleneamino]guanidinium acetate has been synthesized by a four-step procedure. This involved reduction of the Weinreb amide *N*,*N*′-dimethyl-*N*,*N*′-dimethyloxybutane-1,4-diamide-1,2,3,4-$^{13}C_4$ by Dibal-H to give the corresponding unstable dialdehyde which is reacted *in situ* with 4-chloroaniline to form 1-(4-chlorophenyl)-1H-pyrrole-$^{13}C_4$. This pyrrole analogue underwent a Vilsmeyer acylation with $POCl_3$/DMF followed by final reaction with aminoguanidine bicarbonate to produce the desired labelled compound with 99% atom ^{13}C. By using DMF [α-^{14}C] a radio-labelled analogue was synthesized with a specific activity of 60 mCi/mmol. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: *N*-[1-(4-chlorophenyl)-1H-pyrrol-2-yl-$^{13}C_4$-methyleneamino]guanidinium acetate; 1-(4-chlorophenyl)-1H-pyrrole-$^{13}C_4$; succinic acid-$^{13}C_4$; Paal–Knorr reaction

Introduction

Compounds labelled with stable isotopes are routinely used as internal standards in LC–MS assays.[1] When a chlorine atom is present in the molecule, a mass increase of at least $M+4$ is normally required so the parent ion cluster is well separated from that of the non-labelled compound. In the development of a bioanalysis method, the stable labelled analogue **5a** was needed. The present work describes the method developed for the preparation of *N*-[1-(4-chlorophenyl)-1H-pyrrol-2-yl-$^{13}C_4$-methyleneamino] guanidinium acetate (**5a**) using commercially available succinic acid-$^{13}C_4$, suitable for the synthesis of a

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multiply and specifically labelled pyrrole ring. For pharmacological and metabolic studies, the radioactive form **5b** was synthesized starting from the commercially available 1-(4-chlorophenyl)-1H-pyrrole (**3b**) and DMF [α-^{14}C].

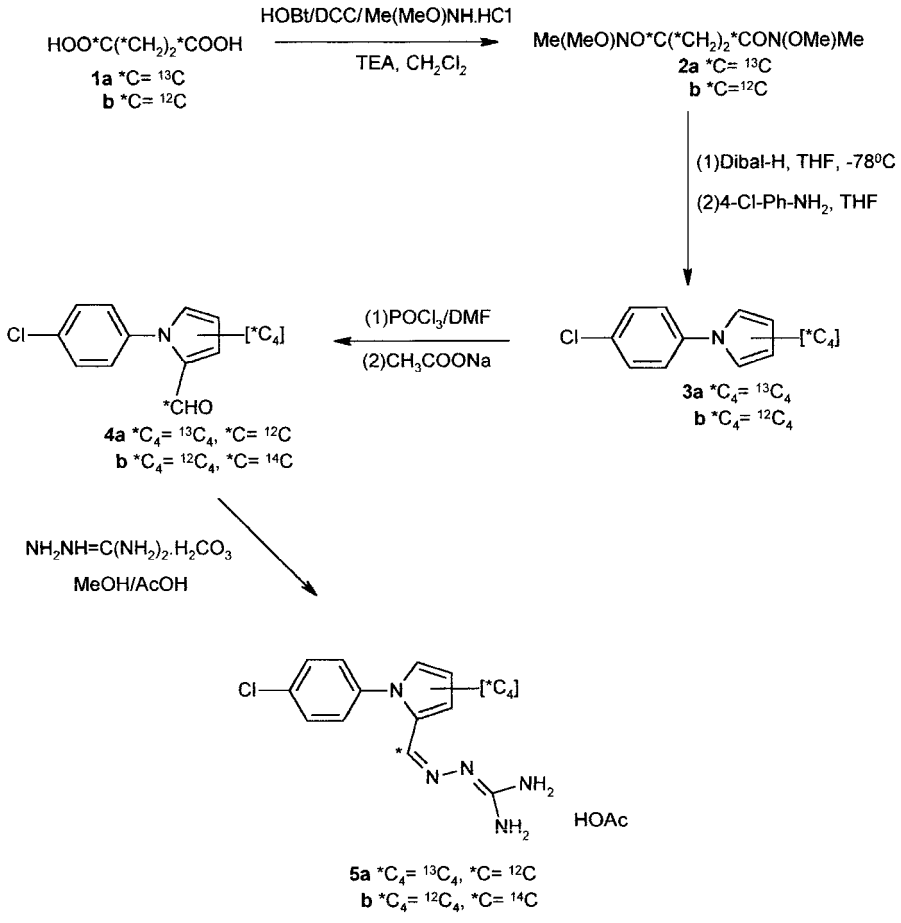
Results and discussion

The synthesis of non-labelled *N*-1[(4-chlorophenyl)-1H-pyrrol-2-yl-methyleneamino]-guanidinium acetate is easily prepared from commercially available 1-(4-chlorophenyl)-1H-pyrrole by a Vilsmeyer acylation,[2] yielding 1-(4-chlorophenyl)-1H-pyrrole-2-carbaldehyde followed by the reaction with the aminoguanidinium bicarbonate.[3] For the synthesis of the labelled analogue **5a** the key step was the labelling of the pyrrole ring.

Our first attempt was to *N*-arylate the commercially available pyrrole-d_5 with 1-chloro-4-iodobenzene. Several reported procedures[4–6] were tried for the *N*-arylation of pyrrole. No reaction occurred either using the NaH[4] or the Cu catalysed[5] methods. The microwave assisted reaction using K_2CO_3/KOH and catalytic amounts of tetrabutylammonium bromide[6] turned out to be sluggish and only 10% of a mixture of the correct compound 1-(4-chlorophenyl)-1H-pyrrole and 1-(4-iodophenyl)-1H-pyrrole was detected by ^{1}H NMR.

The next approach was to take advantage of the Paal–Knorr reaction,[7] which is the most important preparative method for pyrroles. A variety of *N*-substituted pyrroles can be prepared using 2,5-dimethoxytetrahydrofuran as a succinaldehyde equivalent.[8] In our case the idea was to generate *in situ* the labelled dialdehyde starting from the commercially available 1,4-butanediol-$^{13}C_4$. First tried was the oxidation of 1,4-butanediol to the corresponding dialdehyde by using several oxidation procedures (Swern,[9] Dess–Martin,[10] PCC/Al_2O_3[11]) followed by the addition of 4-Cl-aniline. While the Swern[9] and Dess–Martin[10] oxidations failed, the PCC/Al_2O_3[11] method gave the correct product in 30% yield. However, a side product was detected to some extent, which was difficult to separate, and thus no attempts were made to optimize the reaction further.

Finally, an alternative approach starting from succinic acid proved to be successful (Scheme 1). The labelled succinic acid-$^{13}C_4$ (**1a**) was readily transformed to the Weinreb[12] diamide **2a** by using standard coupling reagents in 79% yield after purification. The diamide **2a** was then reduced by Dibal-H followed by *in situ* addition of 4-Cl-aniline. The labelled pyrrole derivative **3a** was obtained in 40% yield after purification. The Vilsmeyer acylation of **3a** with $POCl_3$/DMF was then easily performed yielding compound **4a** in 65% yield after column chromatography. The last step involved the condensation of the aldehyde **4a** with aminoguanidine to afford **5a** in good yield (80%) with 99.9% ^{13}C-atom purity.



Scheme 1.

The synthesis of the radioactive analogue **5b** could be easily accomplished in a two-step reaction. Formylation of the commercially available **3b** with labelled DMF [α-^{14}C] formed the aldehyde **4b** in 84% yield. The standard condensation of **4b** with aminoguanidine gave the desired radio-labelled **5b** in 74% yield with 99% radiochemical purity.

Conclusion

A convenient method was developed for the synthesis of a multiply and specifically labelled *N*-substituted ^{13}C-pyrrole ring starting from commercially available succinic acid-^{13}C. Using this method, the $^{13}C_4$-labelled compound **5a** was efficiently synthesized with 99.9% atom purity. The Vilsmeyer reaction using labelled DMF [α-^{14}C] is the method of choice for the introduction of the radioactive acyl group at position 1 in a pyrrole ring. Thus, the radio-labelled **5b** was easily prepared in 62% overall yield with 99% radiochemical purity.

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. Succinic acid-$^{13}C_4$ (99.9% ^{13}C) was purchased from Aldrich. 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 (^1H) 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. MS analyses were performed on a Micromass Quattro Ultima mass spectrometer.

Synthesis of N-[1-(4-chlorophenyl)-1H-pyrrol-2-yl-$^{13}C_4$-methyleneamino]guanidinium acetate

N,N′-dimethyl-N,N′-dimethyloxysuccinimide-$^{13}C_4$ (2a). To a suspension of **1a** (1.00 g, 8.20 mmol) and methylmethyloxyamine hydrochloride (2.40 g, 24.6 mmol) in dry methylene chloride (80 ml) was added triethylamine (4.15 g, 41.0 mmol). To this mixture was added 1-hydroxybenzotriazole (2.44 g, 18.0 mmol) and the resulting solution stirred at room temperature. *N,N′*-dicyclohexylcarbodiimide (4.06 g, 19.7 mmol) in dry methylene chloride (20 ml) was then added to the reaction mixture and left with stirring overnight at room temperature. The solvent was evaporated and the residue treated with diethyl ether (200 ml). The white precipitate was filtered off and washed several times with diethyl ether. The ethereal solution was evaporated and the crude colourless oil was chromatographed (silica gel, dichloromethane/methanol 20:1) to afford 1.35 g (79%) of the product as a white solid: ^1H NMR δ 3.75 (s, 6 H), 3.20 (br d, 6 H, $J = 1.6$ Hz), 2.79 (dm, 4 H, $J_{C–H} = 125.6$ Hz).

1-(4-chlorophenyl)-1-H-pyrrole-$^{13}C_4$ (3a). Dibal-H (14.1 ml of a 1 M solution in THF, 14.1 mmol) was added dropwise via syringe to a solution of diamide **2a** (1.33, 6.39 mmol) and cooled to −78°C under nitrogen. The mixture was stirred for about 3 h at −78°C under nitrogen. 4-Chloroaniline (3.26 g, 25.6 mmol) in dry tetrahydrofuran (10 ml) was added dropwise via syringe followed by addition of water (5 ml). The reaction mixture was left to warm to room temperature and stirred overnight. The mixture was extracted with diethyl ether (200 ml) and the organic layer washed in turn with saturated citric acid solution (3 × 50 ml), saturated sodium bicarbonate solution (3 × 50 ml), saturated sodium chloride solution (3 × 50 ml), dried (anhydrous sodium

sulphate), and finally the solvent evaporated. The crude product was then purified by silica gel chromatography (petroleum ether/diethyl ether 20:1) to afford 540 mg (46%) of the product as a white solid: ^{1}H NMR δ 7.49–7.30 (m, 4H), 7.06 (dm, 2H, $J_{C-H} = 177$ Hz), 6.42 (dm, 2H, $J_{C-H} = 201$ Hz).

1-(4-chlorophenyl)-1H- pyrrole-$^{13}C_4$ –2-carbaldehyde (4a). To a stirred and ice-bath cooled solution of *N*,*N*-dimethylformamide (256 mg, 3.50 mmol) in 1,2-dichloroethane (5 ml) was added phosphorus oxychloride (537 mg, 3.50 mmol) via syringe under nitrogen. The ice-bath was removed and the mixture stirred at room temperature under nitrogen for 15 min. The reaction mixture was again ice-bath cooled and additional 1,2-dichloroethane (10 ml) was added. A solution of **3a** (530 mg, 2.92 mmol) in 1,2-dichloroethane (5 ml) was added via syringe to the cooled and stirred reaction mixture under nitrogen. The resulting clear solution was refluxed with stirring for 30 min and then cooled to room temperature. A solution of sodium acetate (1.20 g, 14.6 mmol) in water (25 ml) was added and the resulting mixture refluxed with stirring for about 20 min. After cooling to room temperature, the organic layer was separated and the aqueous phase extracted with diethyl ether (3 × 20 ml). The combined organic layer was washed in turn with saturated sodium carbonate solution (2 × 20 ml), saturated sodium chloride (3 × 20 ml), dried (anhydrous sodium sulphate), and finally the solvent was evaporated. The crude product was then purified by silica gel chromatography (petroleum ether/diethyl ether 2:1) to afford 400 mg (65%) of the product **4a** as a white solid: ^{1}H NMR δ 9.59 (d, 1H, $J_{C-H} = 28.1$ Hz), 7.54–7.41 (m, 2H), 7.35–7.25 (m, 2H), 7.12 (dm, 1H, $J_{C-H} = 147$ Hz), 7.06 (dm, 1H, $J_{C-H} = 189$ Hz), 6.44 (dm, 1H, $J_{C-H} = 180$ Hz).

N-[1-(4-chlorophenyl)-1H-pyrrol-2-yl-$^{13}C_4$-methyleneamino]guanidinium acetate (5a). Aminoguanidinium bicarbonate (286 mg, 2.10 mmol) and aldehyde **4a** (400 mg, 1.91 mmol) were suspended in methanol (30 ml). Acetic acid (2 ml) was added and the resulting mixture refluxed for about 5 h. The solvent was evaporated and diethyl ether was added to the resulting yellow oil. After cooling, the product crystallized as an off-white powder (500 mg, 80%): mp165–166°C; ^{1}H NMR (DMSO-d_6) δ 7.79 (d of unresolved d, 1H, $J_{C-H} = 9.4$, $J = 2.8$ Hz), 7.62–7.52 (m, 2H), 7.44–7.30 (m, 2H), 7.04 (dm, 1H, $J_{C-H} = 190$ Hz), 6.69 (dm, 1H, $J_{C-H} = 174$ Hz), 6.30 (dm, 1H, $J_{C-H} = 158$ Hz), 6.15–5.70 (br signal, 4H), 1.81 (s, 3H); MS *m*/*z* (relative intensity) 267 (M^+, 100).

Synthesis of N-[1-(4-chlorophenyl)-1H-pyrrol-2-yl–methylene-^{14}C-amino]guanidinium acetate

1-(4-chlorophenyl)-1H-pyrrole-2-carbaldehyde-^{14}C (4b). The pyrrole **3b** (500 mg, 2.81 mmol), DMF [α-^{14}C] (253 mg, 3.37 mmol) and phosphorus

oxychloride (517 mg, 3.37 mmol) underwent the Vilsmeyer reaction as described for **4a** to yield 480 mg (84%) of the pure product as white crystals identical with an authentical sample.

N-[1-(4-chlorophenyl)-1H-pyrrol-2-yl-methylene-^{14}C-amino]guanidinium acetate (5b). The aldehyde **4b** (480 mg, 2.11 mmol) and aminoguanidinium bicarbonate (287 mg, 2.11 mmol) were reacted as described for **5a**. Purification of the crude product by HPLC (Hypersil BDS C8 3μ (100 × 3.0 mm), 10 mM ammonium acetate + 1% formic acid:acetonitrile (65:35), isocratic, flow rate: 0.3 ml/min) afforded 532 mg (74%) of the product as an off-white powder: mp 166.0–166.5°C; ^{1}H NMR (DMSO-d_6) δ 7.80 (s, 1H), 7.62–7.56 (m, 2H), 7.44–7.33 (m, 2H), 7.06 (dd, 1H, J = 2.4 and 1.6 Hz), 6.75 (dd, 1H, J = 3.6 and 1.6 Hz), 6.29 (t, 1H, J = 3.2 Hz), 6.90–6.10 (br s, 5H), 1.78 (s, 3H). Radioactive **5b** was obtained in 99% radiochemical purity as indicated by HPLC (conditions as above) with a specific activity of 60 mCi/mmol as determined by MS.

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