Synthesis of [^{3}H]FPL 64176, a Potent Calcium Channel Activator

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Summary

Tritium labelled FPL 64176 (1, methyl 2,5-dimethyl-4-[2-(phenylmethyl)benzoyl]-1*H*-pyrrole-3-carboxylate), a potent calcium channel activator with insulinotropic properties was synthesised from the corresponding bromo derivative (3) using tritium gas and Pd/C catalyst. (3) was in turn prepared from methyl 2,5-dimethylpyrrole-3-carboxylate (4) in a one pot procedure. The specific activity of [^{3}H]FPL 64176 was 38 mCi/mmol and radiochemical purity >98%.

Key words: FPL 64176, tritium, calcium channel activator, insulinotropic.

Introduction:

FPL 64176 (1, methyl 2,5-dimethyl-4-[2-(phenylmethyl)benzoyl]-1*H*-pyrrole-3-carboxylate) is a potent activator of the voltage-dependent L-type calcium channel[1]. It has been shown to possess higher efficacy and a mechanism and site of action that are distinct from those of (S)-Bay K 8644, a dihydropyridine calcium channel activator[2,3,4]. FPL 64176 has further been shown to be a potent insulin secretatogogue from the insulin secreting βTC3 cell line *via* opening of Ca^{2+} channels, followed by Ca^{2+}-influx with concomitant rise in intracellular calcium concentration[5]. In order to further study the mechanism by which FPL 64176 acts, a radioligand would be useful. We now report the synthesis of [^{3}H]FPL 64176 (2).

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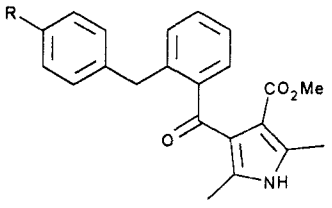
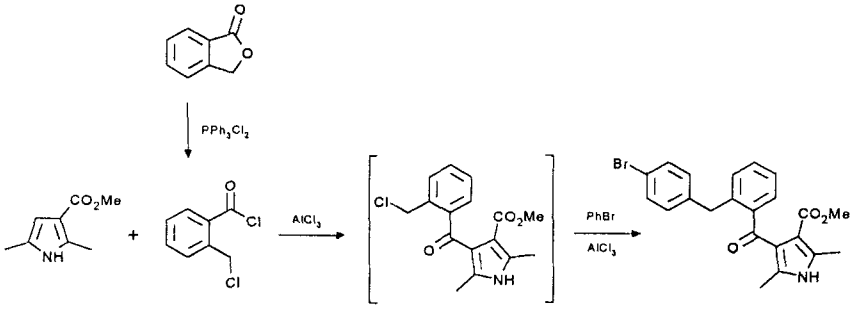


R = H	FPL 64176	1
R = ^{3}H	[^{3}H]FPL 64176	2
R = Br		3

Discussion:

[^{3}H]FPL 64176 (2) was prepared *via* the bromo intermediate (3) using tritium gas. An extremely short synthesis of FPL 64176 has been reported[6], and this method was adopted - with a slight modification - for the the synthesis of the key bromo intermediate, 3, see scheme.

Methyl 2,5-dimethylpyrrole-3-carboxylate (4) was Friedel-Crafts acylated with 2-chloromethylbenzoyl chloride, which had been prepared[7] from phthalide and dichlorotriphenylphosphorane at 180°C without isolation. The unstable 5 thus formed was arylated with bromobenzene *in situ* to give 3 which was isolated by flash chromatography in 19% overall yield.



4 5 3 19%

Scheme

Tritiation of 3 resulted in 2 with a radiochemical purity >98%. HPLC analysis of the reaction mixture showed that no precursor was left after tritiation, resulting in a high specific activity in the product. HPLC purification was not necessary. The specific activity of 38 Ci/mmol indicates, that tritiation also occurs in other parts of the molecule, probably to a large extent in the benzylic position.

Experimental:

TLC was performed on glass plates coated with silica gel 60 F_{254} (Merck). For flash chromatography[8] silica gel 60 (40-63 μm) was used. Evaporations were performed under reduced pressure at 40° C. Solvents were dried with and kept over molecular sieves (4Å). Melting points were obtained on a Büchi 535 apparatus, ^{1}H-NMR spectra were recorded on a Bruker AC 300 MHz spectrometer, with chemical shifts δ measured in ppm downfield from internal standard Me_4Si (δ= 0 ppm). Microanalyses were performed at the mikroanalytical laboratory, Novo Nordisk A/S. Tritiation of **3** was done at Amersham, UK. Determination of total radioactivity was done on a Packard 2000 CA tri-card liquid scintillation analyzer, using 20 ml counting vials and Pico-aqua™ Packard liquid scintillator. HPLC analysis was performed using a Merck HPLC pump L-6200 with a rheodyne injector (20 μl) and a Merck UV-detector L-4000 operating at 254 nm. Separations were accomplished at RT with a C-18 coloumn (250 x 4.6 mm, 5 μm) from Novo Nordisk A/S, using an eluent of methanol and water (85:15). The flow rate was 0.8 ml/min.

Methyl 4-[2-(4-bromophenylmethyl)benzoyl]-2,5-dimethylpyrrole-3-carboxylate, 3.

2.68 g (20 mmoles) Phthalide and 8.48 g (21 mmoles, 80 %) dichlorotriphenylphosphorane was mixed under argon and heated at 180° C for 4 h. The cooled mixture was dissolved in CH_2Cl_2 (20 ml) and 3.25 g (24 mmoles) $AlCl_3$ was added followed by a solution of 3.00 g (19.6 mmoles) methyl 2,5-dimethylpyrrole-3-carboxylate[9] in CH_2Cl_2. After 15 min 3.25 g (24 mmoles) $AlCl_3$ was added and after another 10 min 40 ml bromobenzene and 3.25 g (24 mmoles) $AlCl_3$. Stirring was continued for 15 min. The reaction was quenched by pouring the mixture into water (200 ml). The aqueous phase was extracted with CH_2Cl_2 (200 ml). The combined organic phases were washed with water (3x150 ml), dried ($MgSO_4$), filtered and evaporated. The residue (*ca.* 20 g) was chromatographed over silica gel using CH_2Cl_2:EtOAc 9:1 as eluent. The fractions containing the desired compound were evaporated and crystallized (MeOH). The product was further purified by boiling in methanol and filtering hot, giving 1.54 g (19 %) *methyl 4-[2-(4-bromophenylmethyl)benzoyl]-2,5-dimethylpyrrole-3-carboxylate.* Mp. 208.9-9.5° C. R_f = 0.45 (CH_2Cl_2: EtOAc 9:1). ^{1}H-NMR (300 MHz) δ_H: 2.06 (3H, s), 2.29 (3H,s), 3.09 (3H, s), 4.09 (2H, s), 7.17-7.24 (3H, m), 7.35 (1H, m), 7.44 (2H, d, J=8.3 Hz), 11.5 (1H, bs). Anal.: C, H, N.

Methyl 2,5-dimethyl-4-[2-(4-[^{3}H]-phenylmethyl)benzoyl]-pyrrole-3-carboxylate, [^{3}H]FPL 64176, 2

2 mg (5 μmol) 3 was dissolved in dry THF (1 ml). Triethylamine (2 μl)and the catalyst 10% Pd/C (about 5 mg) was added. The tritiation was run for 1h at room temperature using 5 Ci tritium gas (86 μmol, specific activity 58 Ci/mmol). After tritiation the catalyst was removed, and the mixture evaporated. The product was dissolved in ethanol and evaporated. This was repeated several times, to remove all labile tritium. 2 was isolated with a radiochemical purity >98% determined by radio-HPLC. The specific activity was 38 Ci/mmol, determined by radio-HPLC using reference standard and UV-detection. HPLC-analysis showed that no precursor was left after tritiation. The yield was 172 mCi (4.5 μmol).

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