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$^{1}H-\{^{19}F\}$ NOE difference spectroscopy as a tool for structural assignment of positional isomers in fluorine-substituted analogues of FPL 64176

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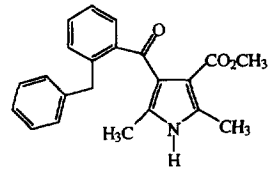
Abstract

Two difluorinated analogues of FPL 64176 (methyl 4-(2-benzylbenzoyl)-2,5-dimethylpyrrole-3-carboxylate) have been synthesized in three steps from 6-fluorophthalide and methyl 2,5-dimethylpyrrole-3-carboxylate. Significant overlap of signals was observed in the aromatic regions of the ^{1}H NMR spectra of these compounds. Using $^{1}H-\{^{1}H\}$ and $^{1}H-\{^{19}F\}$ NOE difference spectroscopy it was possible to identify and assign all aromatic resonances and, based on the NOEs observed, differentiate between the positional isomers and definitely assign the structure of each.

Keywords: $^{1}H-\{^{19}F\}$ NOE difference spectroscopy; FPL 64176

1. Introduction

Voltage-dependent calcium channels provide an important pathway for calcium influx into a variety of tissues. While several distinct types of voltage-dependent calcium channels have been described, L-type channels are particularly important since they modulate excitation–contraction coupling in the cardiovascular system. While a variety of structural classes have been reported to block calcium entry through these channels [1], relatively few compounds have been reported to have the opposite effect [2]. Recently methyl 4-(2-benzylbenzoyl)-2,5-dimethylpyrrole-3-carboxylate (**1**) was described as a potent activator of L-type calcium channels [3–5]. We have subsequently presented a very short synthesis of **1** which employed 2-chloromethylbenzoyl chloride, prepared from phthalide and triphenylphosphine dichloride, as a Friedel–Crafts acylating agent [6]. In an attempt to extent our method to derivatives containing a fluorine atom in the proximal aromatic ring, we reacted 6-fluorophthalide and triphenylphosphine dichloride. Surprisingly, we observed that this reaction induced an isomerization of the fluorine substituent. The structural consequence of this isomerization were elucidated by NMR techniques which included a combination of $^{1}H-\{^{1}H\}$ and $^{1}H-\{^{19}F\}$ NOE difference experiments.



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2. Results and discussion

By analogy to our previously reported synthesis of FPL 64176, 6-fluorophthalide (**2**) [7] and triphenylphosphine dichloride were heated at 190 °C (see Scheme 1). Kugelrohr distillation afforded approximately a 1:3 mixture based on the integration of NMR signals of 2-chloromethyl-4-fluorobenzoyl chloride (**3**) and 2-chloromethyl-5-fluorobenzoyl chloride (**4**). Structural assignments of these positional isomers were accomplished based on NMR data which showed well resolved resonances for each component. The ^{1}H NMR resonances for the aromatic protons were first differentiated based on $^{1}H-^{1}H$ and $^{1}H-^{19}F$ coupling constants and then $^{1}H-^{1}H$ NOE difference data obtained by preirradiation of the methylene protons were used to assign substitution patterns. Thus, for **3**, a large NOE was observed from the methylene protons to an aromatic signal with ortho $^{1}H-^{19}F$ and meta

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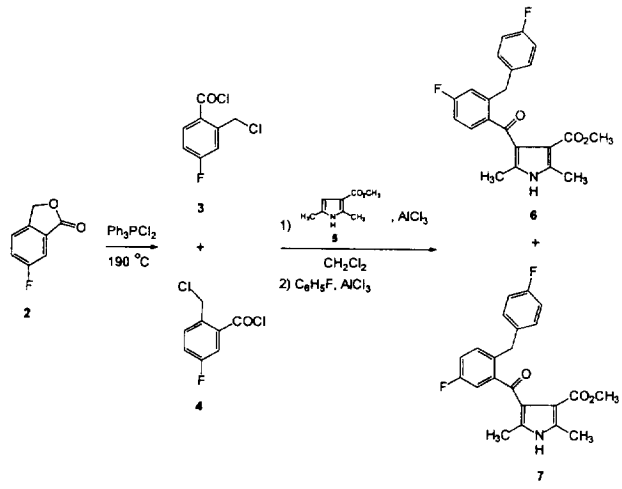

Scheme 1.

^1H–^1H couplings, while for **4** a large NOE to an aromatic signal with meta ^1H–^{19}F and ortho ^1H–^1H couplings was observed. These NOEs are consistent with the structures shown. While the structural integrity of the starting material **2** was examined by NMR and shown to be a single set of resonances consistent with the single isomer drawn, no other attempts were made to try to elucidate the mechanism of this isomerization.

Feeling that acid chlorides **3** and **4** would be difficult to separate, we reacted this mixture sequentially with methyl 2,5-dimethylpyrrole-3-carboxylate (**5**)/aluminum chloride and fluorobenzene/aluminum chloride. Purification of the resulting mixture by flash chromatography [8] afforded low yields of methyl 2,5-dimethyl-4-[2-(4-fluorobenzyl)-4-fluorobenzoyl]pyrrole-3-carboxylate (**6**) and methyl 2,5-di-methyl-4-[2-(4-fluorobenzyl)-5-fluorobenzoyl]pyrrole-3-carboxylate (**7**). Structural assignments of each isomer were then undertaken by NMR. An initial attempt to assign regiochemistry based on the predicted chemical shifts of the aromatic protons ortho to the keto-carbonyl was futile due to the inability to assign resonances because of severe overlap of the seven aromatic protons in each structure. It was also felt that structural assignments made based on predicted chemical shifts would be tentative at best because of the uncertainty in predicting chemical shifts in these compounds. It was therefore necessary to use a combination of ^1H–{^1H} and ^1H–{^{19}F} NOE difference spectroscopy to identify and assign various resonances in the overlapped aromatic region and unequivocally assign the regiochemistry.

While ^1H–^{19}F NOE experiments were first reported more than 25 years ago [9], few applications have been reported in recent years [10–16] even though this is a direct and powerful method of structure elucidation. Recent examples that have been reported have often involved the editing of ^1H spectra of fluorine-labeled macromolecules via ^1H-{^{19}F} NOEs [10] or the utilization of fluorine-labeled ligands to study protein–ligand systems via intermolecular transferred NOEs [16]. The limited application of ^1H–^{19}F NOE experiments is presumably due to hardware limitations of many older spectrometers. For the ^1H–{^{19}F} NOE difference experiments used in this study two hardware requirements are worthy of discussion.

First, this experiment requires an NMR probe which is capable of being tuned to both ^1H and ^{19}F at the same time. Many older NMR probes have high frequency coils which can be tuned to proton or fluorine, but not both at the same time. Newer generation NMR probes often have two high frequency coils which are independently tuned, thus overcoming this problem, or, in our case, we used a four-nuclei probe with a single high frequency coil with a double tuned circuit for ^1H and ^{19}F. The second hardware consideration is rf generation. Since the spectrometer we used for these experiments was a single broadbanded system, it was necessary to run these experiments in the "reverse" mode. That is, the ^1H decoupler was used as the observe transmitter and the broadband transmitter as the ^{19}F decoupler transmitter.

Standard steady-state NOE difference pulse sequences were used for both the ^1H–{^1H} and ^1H–{^{19}F} NOE difference experiments. The preirradiation time for both experiments was not optimized but conservatively set at ten seconds for both experiments. It is believed this time can be shortened considerably without significant degradation of the NOE enhancements observed. Typically 64–256 transients were obtained with total experiment times of several hours or less, demonstrating the simplicity of these experiments. Like ^1H–{^1H} NOEs, ^1H–{^{19}F} NOEs can be large (theoretical maximum enhancement 53% [9]) but typically are much smaller due to instrumental, environmental and sample considerations. Unlike ^1H–{^1H} NOEs, with ^1H–{^{19}F} experiments there are no problems with incomplete saturation since nearby signals are typically not an issue.

The ^1H–{^1H} and ^1H–{^{19}F} NOE difference spectra used to assign the regiochemistry of **6**, as well as a reference ^1H survey spectrum, are shown in Fig. 1. Seven aromatic protons

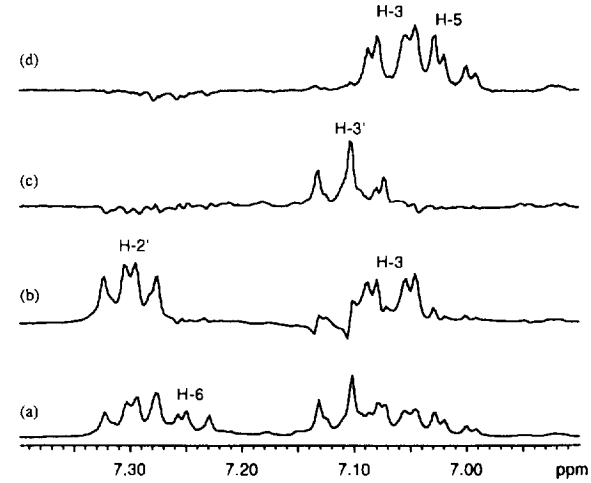

Fig. 1. Aromatic region of ^1H NMR spectra of **2**: curve a. survey, spectrum; curve b, ^1H–{^1H} NOE difference spectrum after preirradiation of methylene protons; curve c, ^1H–{^{19}F} NOE difference spectrum after preirradiation of ^{19}F δ − 116.8; curve d, ^1H–{^{19}F} NOE difference spectrum after preirradiation of ^{19}F δ − 109.9; see Fig. 2 for number scheme.

are observed in the region shown between 6.99 and 7.34 ppm. Initially, assignments were not possible due to overlap of signals but integration did indicate four protons between 6.99 and 7.14 ppm with the remaining three between 7.22 and 7.34 ppm. Starting with this information, a $^1H-\{^1H\}$ NOE difference spectrum (Fig. 1, spectrum b) was obtained preirradiating the methylene protons (δ 4.14) to identify protons H-2′ and H-3 (see Fig. 2 for number scheme). A doublet of doublets ($J = 10$ and 2.5 Hz) was observed at 7.06 ppm which was assigned as H-3 based on the large $^{19}F-^1H$ ortho-coupling (10 Hz) and small $^1H-^1H$ meta-coupling (2.5 Hz). H-2′ was assigned as the doublet of doublets at 7.29 ppm with $^1H-^1H$ ortho-coupling (8.5 Hz) and $^{19}F-^1H$ meta-coupling (5.5 Hz). The remaining protons were then assigned based on the two $^1H-\{^{19}F\}$ NOE difference spectra shown. Preirradiation of the fluorine signal at −116.8 ppm (Fig. 1, spectrum c) led to observation of H-3′ as a triplet ($^{19}F-^1H$ and $^1H-^1H$ ortho-couplings are both 8.5 Hz), while preirradiation of the fluorine signal at −109.9 ppm (Fig. 1, spectrum d) led to enhancement of H-3 and H-5. The identification of H-3 enhanced by both preirradiation of the methylene protons and the fluorine at −109.9 ppm unequivocally establishes the regiochemistry of **6** as the isomer drawn. H-5 was observed as a partially resolved triplet of doublets at 7.02 ppm ($J = 8.5$ and 2.5 Hz, confirmed by $^1H-^{19}F$ decoupling experiments, not shown) with 8.5 Hz $^{19}F-^1H$ and $^1H-^1H$ ortho-couplings and 2.5 Hz $^1H-^1H$ meta-coupling. The remaining resonance, not enhanced in any of the NOE experiments, was H-6 observed at 7.25 ppm as a partially resolved doublet of doublets with 8.5 Hz $^1H-^1H$ ortho-coupling and 6 Hz $^{19}F-^1H$ meta-coupling.

A similar analysis of **7** gave NOEs consistent with its claimed regiochemistry. Specifically, preirradiation of the methylene protons (δ 4.05) led to observation of NOEs to H-2′ (δ 7.3–7.2, m) and H-3 (δ 7.3–7.2, m), while preirradiation of the fluorine signal at −116.6 ppm led to enhancement of H-4 (δ 7.21, td, $J = 8.5$ and 2.5 Hz) and to H-6 (δ 6.93, dd, $J = 9.5$ and 2.5 Hz). Finally, preirradiation of the fluorine signal at −117.0 ppm led to enhancement of H-3′ (δ 7.08, t, $J = 9$ Hz). These NOEs and coupling constants are only consistent with the positional isomer shown. It is interesting to note that for **7** H-6, ortho to the keto-carbonyl, is the farthest upfield of the aromatic protons occurring at 6.93 ppm. This is presumably due to a solution conformation where H-6 is rotated into the shielding cone of the carbonyl, or is situated in a shielding region above the pyrrole. This observation demonstrates the difficulty in assigning structures based on predicted chemical shifts and reinforces the utility of direct methods such as the NOE experiments presented. The NOEs observed for both **6** and **7** are summarized in Fig. 2.

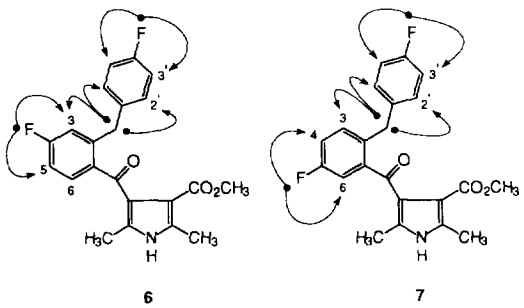

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Fig. 2. NOEs (depicted by arrows) observed in $^1H-\{^1H\}$ and $^1H-\{^{19}F\}$ NOE difference spectra for **6** and **7**.

3. Conclusions

As a result of an unexpected isomerization in the first step of a three-step synthesis of a difluorinated analogue of the calcium L-channel agonist FPL 64176, two fluorine regioisomers were isolated and characterized by NMR techniques. Significant overlap of signals was observed in the aromatic regions of the ^1H NMR spectra of these compounds. Using $^1H-\{^1H\}$ and $^1H-\{^{19}F\}$ NOE difference spectroscopy it was possible to identify and assign all aromatic resonances and, based on the NOEs observed, differentiate between the positional isomers and definitively assign the structure of each.

4. Experimental details

4.1. General

Melting points were determined in open capillaries on a Thomas However apparatus and are uncorrected. IR spectra were recorded using a Mattson Galaxy 5020 FT-IR spectrophotometer. MS data were collected at 70 eV on a Finnigan MAT 4600 mass spectrometer. The NMR spectra were recorded at 25 °C on a Varian Unity-300 spectrometer. The chemical shifts are reported in parts per million versus internal tetramethylsilane for ^1H and external fluorotrichloromethane for ^{19}F. NOE difference spectra were obtained using standard Varian pulse sequences (D1 = 10 s (not optimized), AT = 1 s, decoupler power sufficient to completely saturate the signal of interest). The NMR probe used was a Varian 4-nucleus $^1H/^{19}F/^{13}C/^{31}P$ Auto-NMR™ probe. Combustion analysis were performed using a Perkin-Elmer Model 2400 elemental analyzer.

4.2. Methyl 2,5-dimethyl-4-[2-(4-fluorobenzyl)-4-fluorobenzoyl]pyrrole-3-carboxylate (6) and methyl 2,5-dimethyl-4-[2-(4-fluorobenzyl)-5-fluorobenzoyl]-pyrrole-3-carboxylate (7)

A mixture of 6-fluorophthalide [7] (5.00 g, 32.9 mmol, ^1H NMR ($CDCl_3$): δ 7.58 (dd, 1H, $J = 7$ and 2.5 Hz), 7.49 (ddm, 1H, $J = 8.5$ and 4.5 Hz), 7.41 (td, $J = 8.5$ and 2.5 Hz), 5.31 (s, 2H); ^{19}F NMR ($CDCl_3$) δ −112.1) and triphenylphosphine dichloride (15.6 g, 38.6 mmol, 80%) was heated at 190 °C for 5 h. The reaction was then kugelrohr distilled affording approximately a 1:3 mixture of 2-chloro-

methyl-4-fluorobenzoyl chloride (**3**) and 2-chloromethyl-5-fluorobenzoyl chloride (**4**) as a brown oil: 3.87 g (57%), bp 190 °C (3 mm); ^{1}H NMR ($CDCl_3$): minor isomer **3** δ 8.36 (dd, 1H, J = 9 and 5.5 Hz), 7.42 (dd, 1H, J = 9 and 2.5 Hz), 7.19 (ddd, 1H, J = 9, 7.5 and 2.5 Hz), 4.91 (s, 2H); major isomer **4** δ 7.96 (dd, 1H, J = 9 and 2.5 Hz), 7.61 (dd, 1H, J = 8.5 and 5.5 Hz), 7.35 (ddd, 1H, J = 8.5, 7.5 and 2.5 Hz), 4.87 (s, 2H); ^{19}F NMR ($CDCl_3$): minor isomer **3** δ −101.1 (ddd, J = 9, 7.5 and 5.5 Hz); major isomer **4** δ −110.8 (m). A portion of this mixture (1.56 g, 7.53 mmol) was dissolved in CH_2Cl_2 (25 ml). To this solution was added $AlCl_3$ (1.17 g, 8.77 mmol). A solution of methyl 2,5-dimethylpyrrole-3-carboxylate (1.15 g, 7.51 mmol) in CH_2Cl_2 (10 ml) was added dropwise over approximately 4 min. After stirring 15 min, fluorobenzene (10 ml) and $AlCl_3$ (2.05 g, 15.4 mmol) were added. After 30 min, the reaction was poured into H_2O (100 ml) and the resulting solution was extracted with CH_2Cl_2 (3 × 150 ml). The combined extracts were dried over $MgSO_4$ and then evaporated. Purification by flash chromatography [8] (30% EtOAc/hexane) afforded two products. Crystallization of the lower R_f product from CH_3OH afforded **6** as a colorless solid: 0.138 g (5%), mp 221–222 °C; ^{1}H NMR (dmso-d_6): δ 11.60 (bs, 1H), 7.34–7.22 (m, 3H), 7.14–6.99 (m, 4H), 4.14 (s, 2H), 3.15 (s, 3H), 2.31 (s, 3H), 2.08 (s, 3H); ^{19}F NMR (dmso-d_6): δ −116.8 (m, 1F) −109.9 (m, 1F); (IR (KBr): 1670, 1650 and 1225 cm^{-1}; MS (EI): m/z (% relative intensity) 383 (M^+, 10), 368 (30), 336 (100). Analysis calculated for $C_{22}H_{19}F_2NO_3$: C, 68.92; H, 4.99; N, 3.65. Found: C, 68.69; H, 5.07; N, 3.56. Crystallization of the higher R_f product from CH_3OH afforded **7** as a colorless solid: 0.203 g (7%), mp 195–200 °C; ^{1}H NMR (dmso-d_6): δ 11.65 (bs, 1H), 7.31–7.18 (m, 4H), 7.08 (t, 2H, J = 9 Hz), 6.93 (dd, 1H, J = 9 and 2.5 Hz), 4.05 (s, 2H), 3.14 (s, 3H), 2.31 (s, 3H), 2.10 (s, 3H); ^{19}F NMR (dmso-d_6): δ −116.6 (m, 1F), −117.0 (m, 1F); IR (KBr): 1675, 1650, 1245 and 1200 cm^{-1}; MS (EI): m/z (% relative intensity) 383 (M^+, 30), 368 (30), 336 (100). Analysis calculated for $C_{22}H_{19}F_2NO_3$: C, 68.92; H, 4.99; N, 3.65. Found: C, 68.53; H, 5.04; N, 3.58%.

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