Synthesis, Spectral Studies and Tritiation of the Cannabinoid Antagonist SR141716A

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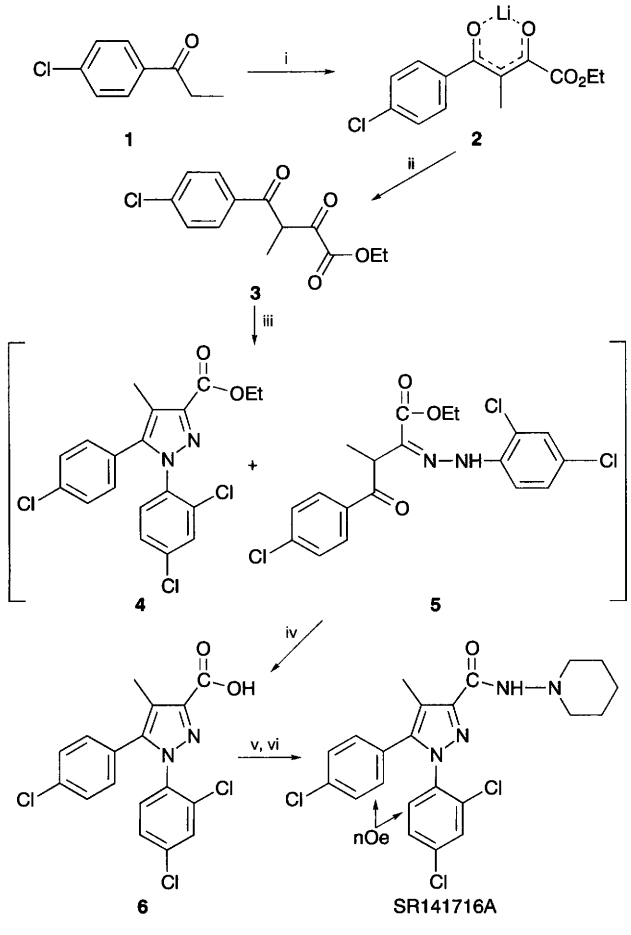
An efficient synthesis of the cannabinoid antagonist SR141716A is presented and the structure is established by nOe experiments; tritiated SR141716A is synthesized by a novel sequence of metallation–iodination–tritiation and the labelled site is shown by tritium NMR and tritium–hydrogen nOe experiments.

Recently, the diarylpyrazole hydrazide SR141716A was reported to be the first antagonist for the cannabinoid receptor.[1] This long-awaited ligand is an important tool for studies that will lead to a better understanding of this neurochemical system represented by the receptor.[2,3] Such studies are greatly facilitated by the availability of the corresponding tritiated radioligand. Towards these ends we have synthesized a tritiated SR141716A by an approach that involves metallation, iodination, and subsequent catalytic reduction. Prior to this, a synthesis of SR141716A was developed since neither the method of synthesis nor a structural proof had been provided in the published reports.[4]

The synthesis of SR141716A from commercially available fragments is shown in Scheme 1. Condensation of 4-chloropropiophenone **1** with diethyl oxylate in the presence of lithium hexamethyldisilazamide[5] afforded the solid lithium salt **2**. Acidification of **2** to provide **3** followed by condensation with 2,4-dichlorophenylhydrazine by heating at reflux gave a 40% yield of the pyrazole ester **4** and a 60% yield of the imine **5**, which were readily separated due to solubility differences between the two compounds in the reaction mixture.[6,7] Further heating of **5** failed to generate more **4**, indicative of a non-reactive *anti* configuration between the anilino nitrogen and the 4-keto group. When **5** or the reaction mixture containing **4** and **5** were heated with ethanolic sodium hydroxide,[8] the pyrazole acid **6** was obtained. The cyclization proceeded *via* base-promoted isomerization about the imine double bond to afford a reactive *syn* relationship between the anilino nitrogen and the 4-keto group. *In situ* hydrolysis of the ester provided acid **6**, which was obtained in a sufficiently pure state to be used in the subsequent step. The acid **6** was converted to the corresponding acid chloride with thionyl chloride[7,9] which was treated, without purification, with 1-aminopiperidine to provide pure SR141716A by crystallization and chromatography of the mother liquor residue in 15% overall yield.†



Scheme 1 *Reagents and conditions*: i, EtO_2CCO_2Et, Li-HMDS; ii, HCl; iii, 2,4-dichlorophenylhydrazine, EtOH, heat; iv, NaOH; v, $SOCl_2$; vi, 1-aminopiperidine, Et_3N

The above condensation of **3** with the hydrazine has the potential to afford an isomeric 1,3-diarylpyrazole as well as the desired 1,5-diarylpyrazole **4**. The latter is usually preferred due to initial attack of the hydrazine at the more electrophilic 2-carbonyl of 2,4-diketo esters.[5,10] A spectral determination of the regiochemistry by comparison of contrasting spectral differences[5,10] of the two isomers was not possible since a second pyrazole isomer was not observed in the condensation step. However, the proximity of the two aryl rings in the 1,5-isomer suggests that a nuclear Overhauser effect (nOe) interaction would exist between the *ortho* protons on the respective aryl rings, thus identifying that isomer. No such effect would be anticipated for the 1,3-isomer. The NMR spectra of the aromatic region of the final product or of the ester precursor in $CDCl_3$ or C_6D_6 were not sufficiently resolved to allow a definitive experiment. However, a spectrum of the compound in MeOD revealed complete resolution of all aromatic resonances. The nOe spectrum clearly demonstrated the expected nOe effect and thereby rigorously identified the final product as the 1,5-diphenylpyrazole SR141716A.

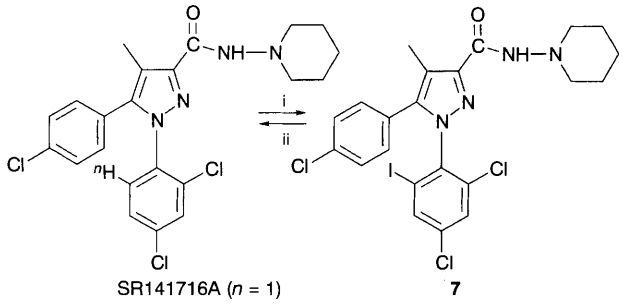
Tritium labelling of the ligand in the preferred final step of its preparation involved the synthesis of a derivative that could be reduced back to the parent compound with a tritium source. The choice of reductive dehalogenation as the tritiation approach, which was to be conducted in the presence of chlorine atoms that were to be maintained in the product, required reducing the significantly more reactive iodide. The rapid reduction of an iodo substituent offers greater selectivity and higher tritium incorporation than could be expected with the more commonly used bromo substitution. However, iodination of an aryl ring, typically by an iodonium reagent, is often more problematic than bromination. In this paper we join a metallation–iodination sequence with catalytic deiodination as a facile tritiation method.

The *N*-substituted 2,4-dichlorophenyl ring with an available *ortho* hydrogen (6-H) opened the possibility for the facile introduction of iodine into that site by directed *ortho* metallation[11] followed by trapping with an iodine source. In practice, SR141716A was metallated (−15 °C, Et_2O) with *n*-butyllithium and quenched with 1-chloro-2-iodoethane to afford the 6-iodophenyl derivative **7** (Scheme 2). The compound was identified by ^1H NMR (MeOD), which exhibited only two *meta*-coupled doublets for the trihalo ring (δ 7.94 and 7.66, J = 2.2 Hz), and MS (DIP) (*m/z* 588 M^+, 84 base). Reduction of **7** under a deuterium atmosphere in the presence of 10% Pd/C (10% *m/m*) and Et_3N (25 °C, Et_2O) afforded deuterium-labelled SR141716A (93% ^2H) that was identical (HPLC, ^1H NMR, MS)

in all other respects with SR141716A. No over-reduced product was produced.

Tritiation under similar conditions with carrier free tritium gas afforded [^{3}H]-SR141716A as predominantly a single component (HPLC, βRAM). HPLC purification afforded the >99% pure ligand at a specific activity of 22.4 Ci mmol^{-1} as determined by UV quantitation and scintillation counting. The proton-decoupled ^{3}H NMR spectrum (MeOD) exhibited one singlet at δ 7.50 for the 6-^{3}H on the 2,4-dichlorophenyl ring, thus identifying the single site of label incorporation. The ^{1}H NMR spectrum of this compound was also consistent with tritiated SR141716A. Furthermore, a tritium–proton nOe spectrum of the ligand demonstrated an enhancement of the 2,6-protons of the 4-chlorophenyl ring, as observed for the proton–proton nOe of unlabelled SR141716A. A bimolecular counterpart to this intramolecular nOe could be applied to studies of the site of interaction of this ligand with its cloned receptor or its relevant fragments thereby adding to the understanding of this pivotal neurochemical interaction.[12]

The synthesis of SR141716A from commercially available fragments by easily conducted chemistry without the need for chromatography or other purification procedures for the intermediates recommend this route for the preparation of this important compound. This first radioligand of the new cannabinoid antagonist is expected to be a valuable tool in pharmacological studies of this emerging neurochemical system. The presentation of the metallation–iodination–catalytic reduction sequence as a facile procedure for tritium labelling enables and suggests its potential wider application to other structures that are amenable to metallation and trapping with the preferred halogen iodine.



SR141716A (*n* = 1) **7**

[^{3}H]SR141716A (*n* = 3)

Scheme 2 *Reagents and conditions*: i, Bu^{n}Li, Et_2O, −15 °C, $ClCH_2CH_2I$; ii, $^3H_2$, 10% Pd/C, Et_2O

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Footnote

† *Selected data* for SR141716A: ^{1}H NMR (MeOD, 250 MHz *J*/Hz) δ: 7.56 (d, *J* 2.2, Ar 3-H), 7.54 (d, *J* 8.5, Ar 6-H), 7.45 (dd, *J* 8.5, 2.2, Ar 5-H), 7.36 (d, *J* 8.5, Ar′ 3,5-H), 7.19 (d, *J* 8.6, Ar′ 2,6-H), 2.83 [br t, *J* 5.3, N-$(CH_2)_2$], 2.30 (s, CH_3), 1.73 [br p, *J* 5.6, N-$(CH_2CH_2)_2$], 1.44 [br m, N-$(CH_2CH_2CH_2)$]; mp 157–159 °C (lit.[4] 154–156 °C); HRMS calc. 462.0781, obs. 462.0783; CHNCl with 0.06%.

All new compounds have been characterised by spectroscopic methods.

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