JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 589–596.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.952

Research Article

Tritiation of the cannabinoid receptor antagonist SR144528 involving lithium aluminum tritide reduction; assessment of the kinetic isotope effect by ^{3}H-NMR

Herbert H. Seltzman*, Matthew C. Foster, Christopher D. Wyrick, Jason P. Burgess and F. Ivy Carroll
Center for Organic and Medicinal Chemistry, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709, USA

Summary

The cannabinoid receptor antagonist SR144528 was synthesized by an approach that enabled the incorporation of high specific activity tritium label while circumventing the lability of the target compound to catalytic hydrogenation. Lithium aluminum tritide of less than maximum specific activity was employed to introduce tritium, resulting in an H/T incorporation indicative of no kinetic isotope effect for the hydride/tritide reduction of a methyl benzoate. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: SR144528; lithium aluminum tritide; KIE; ^{3}H-NMR

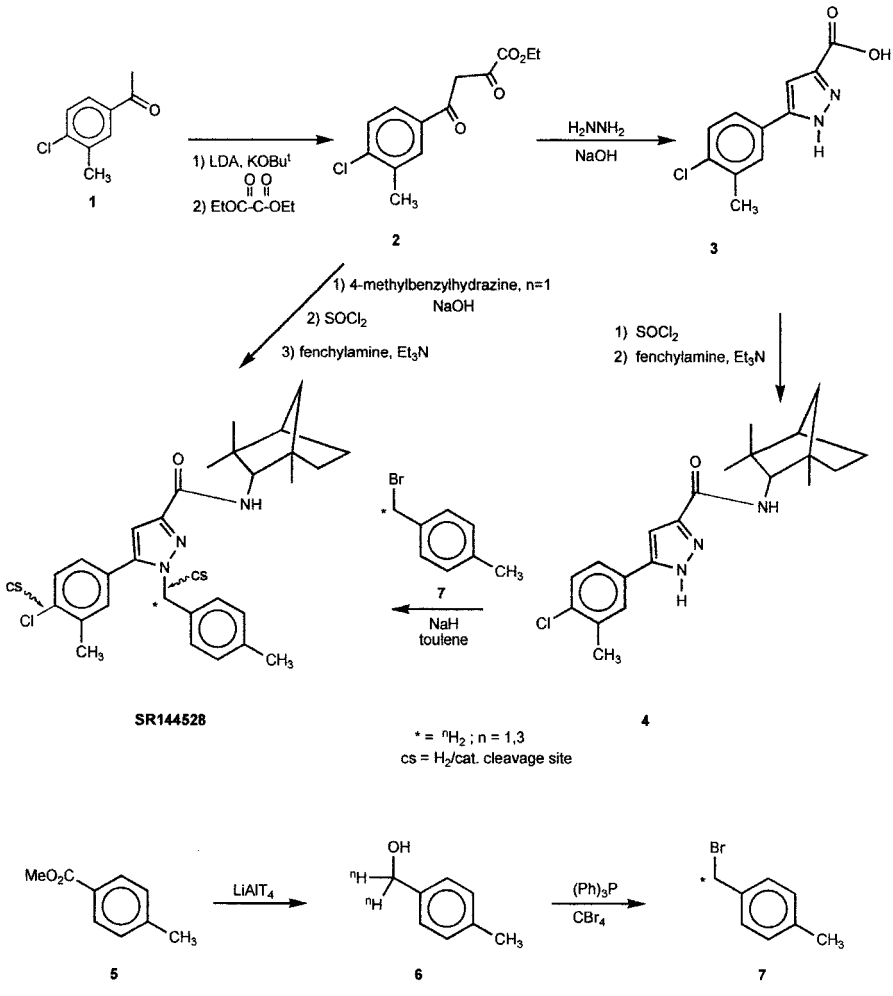
Introduction

The pyrazole carboxamide SR144528 is a selective antagonist at the peripheral cannabinoid CB_2 receptor.[1,2] The potential value of this compound to researchers for pharmacological studies of the CB_2 receptor[3] and its potential role in immune function prompted the synthesis of the tritiated compound. Prior to tritium labelling, the compound was synthesized in unlabelled form by two routes (Scheme 1) that differ in some detail from that reported by Sanofi.[4] The first, followed our reported approach to the synthesis of the Sanofi central cannabinoid CB_1 receptor antagonist SR141716 employing a benzyl hydrazine in a convergent synthesis.[5,6] The second route, reserved the benzylation as the last step in the synthesis for its application to the tritium labeling.

*Correspondence to: Herbert H. Seltzman, Center for Organic and Medicinal Chemistry, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709, USA. E-mail: hhs@rti.org

Contract/grant sponsor: National Institutes on Drug Abuse; contract/grant number: N01DA-6-7054
Contract/grant sponsor: National Institutes of Health; contract/grant number: N01DA-6-7054

Received 14 March 2005
Revised 16 March 2005
Accepted 17 March 2005



Scheme 1.

The unlabeled SR144528 from the first route was characterized by ^{1}H-NMR and MS analyses. Additionally, the product exhibited a nuclear Overhauser effect (nOe) interaction between the benzylic methylene and the 2- and 6-protons of the aryl chloride ring in MeOD. This through space interaction is only possible with the desired 1-benzylic-5-aryl isomer, thus rigorously identifying and distinguishing the major isomer from the minor 1-benzylic-3-aryl isomer. The synthesis produced an 85:15 ratio of the 1,5:1,3 isomers which were separated chromatographically and by recrystallization at the end of the synthesis.

Tritium labeling by catalytic reduction of an unsaturated or further halogenated analog of SR144528 is potentially problematic since cleavage of the chloride or benzyl fragments could occur producing significant radiochemical by-products that could be difficult to remove. In support of this

concern, a sample of SR144528 that was stirred under a deuterium atmosphere over 10% Pd/C in ethanol for 2 h consumed more than half of the material through cleavage of either the chlorine, the benzyl moiety, or both the chlorine and the benzyl moiety (HPLC-electrospray MS) (see Scheme 1).

While selectivity of the reduction of a bromo- or iodo-aryl analog of SR144528 might prove fruitful, we sought an approach that would circumvent the above hazards. This involved the benzylation of the des benzyl analog of SR144528 (**4**) with tritium-labeled 4-methylbenzyl bromide **7** as shown in route 2. Thus, treatment of the diketone **2** with hydrazine and subsequently sodium hydroxide in refluxing ethanol gave the pyrazole acid **3** in 91% yield and of suitable purity for use in the next step after simple extractive workup. Conversion to the acid chloride (92%) and treatment with (-)-fenchylamine[7–9] afforded the fenchylamide **4** in 76% yield and 97% purity (HPLC) by extractive workup. A benzylation with commercially available 4-methylbenzyl bromide mediated by NaH in toluene (140°C, 5 h) following the method used for benzylating the methyl ester of **3**[4] gave SR144528 in 47% yield comparable (HPLC, TLC, NMR) to that produced via route 1. This approach also has the advantage of affording a lesser percentage of the isomeric 3-aryl isomer (6%) than did the benzyl hydrazine approach.

Applying route 2 to the synthesis of tritium-labeled SR144528 started with the production of lithium aluminum tritide produced by the action of tritium gas on *n*-butyllithium and subsequent treatment of the lithium tritide with aluminum bromide as described.[10] Addition of excess methyl 4-methylbenzoate **5** to the lithium aluminum tritide afforded tritiated 4-methylbenzyl alcohol **6**. Bromination with triphenylphosphine and carbon tetrbromide[11,12] provided the tritiated 4-methylbenzyl bromide **7** which was chromatographed and used to benzylate **4** as described above. Chromatography afforded tritiated SR144528 that was chromatographically identical (HPLC B-RAM, UV; TLC-radioscan) to unlabeled SR144528. The specific activity was determined by radioactivity counting and UV quantitation to be 32.5 Ci/mmol.

A proton decoupled ^{3}H-NMR spectrum (533 mHz) of the product showed major and minor singlets at 5.34 δ and 5.36 δ, respectively, as the only resonances. This is indicative of tritiation only in the benzylic position as anticipated. The proton-coupled ^{3}H-NMR spectrum exhibited the 5.34 δ resonance as a singlet and the 5.36 δ resonance as a doublet ($J = 16.4$ Hz) (area ratio 66:50) indicative of CT_2 and CHT, respectively. This, in combination with the 32.5 Ci/mmol specific activity indicates that [^{3}H]SR144528 is a 32:49:19 mixture of CT_2:CHT:CH_2.† Using excess methyl 4-methylbenzoate and complete consumption of the $LiAl^{*}H_4$ to give a specific activity of 32.5 Ci/mmol indicates the percent tritium incorporation of the $LiAl^{*}H_4$ to be 56% (32.5/57.5). A calculation for a random incorporation (i.e. no kinetic isotope effect, KIE) of 56% tritide $LiAl^{*}H_4$ affords a 31:50:19 ratio.‡ Therefore,

comparing the observed ratio (based on ^{3}H-NMR integration) to the calculated ratio suggests a negligible KIE for the lithium aluminum tritide/hydride reduction of the methyl benzoate.[§]

†^{3}H-NMR peak	CT_2	CTH/CHT	CH_2	
^{3}H peak area	66	50	0	
$C^{*}H_2$ mole ratio	33	50	0	
$C^{*}H_2$ mol%	33/83	50/83		
	39.8	60.2		
32.5 Ci/mmol =	(57.5)39.8 +	(28.8)60.2 +	(0)X;	$X = 23.8$
	39.8 +	60.2 +	X	
total mmol = 123.8 =	39.8 +	60.2 +	23.8	
mol%$C^{*}H_2$ = $C^{*}H_2$/123.8 =	32	49	19	

‡H/T Combinations	CTT	CTH	CHT	CHH
Probability of incorporation (from 56% $LIAL^{*}H_4$)	56×55	56×44	44×56	44×43
Molar ratio (calc)	31	25	25	19

Conclusions

A synthesis of tritium-labeled SR144528 was developed to circumvent the lability of the target compound to hydrogenolysis. The synthetic approach involved tritiation via lithium aluminum tritide reduction of a methyl benzoate. The 56% labeled lithium aluminum tritide that was prepared resulted in an incorporation that was determined by ^{3}H-NMR to be the same as a random introduction of hydride and tritide. This is consistent with no kinetic isotope effect in the $LiAl^{*}H_4$ reduction of the methyl benzoate.

Experimental

The preparation of lithium aluminum tritide was conducted on an IN/US Systems Inc. Tri-Sorber Model TS-1000 tritiation manifold. ^{3}H-NMR spectra were acquired on a Bruker AMX-500 operating at 500.13 MHz for ^{1}H and 533.4 MHz for ^{3}H. Spectra were acquired at ambient temperature using a

[§]The hydride is suspected to have derived from trace water in the production of lithium tritide. Trace water, possibly introduced with TMEDA, would react with *n*-BuLi to generate LiOH which in turn would react with LiT to generate HT. Alternatively, LiT could react with water if it is generated faster than water is consumed by *n*-BuLi. The HT would then reduce *n*-BuLi and form LiH and LiT which are subsequently converted to lithium aluminum tritide/hydride thus accounting for the production of mixed incorporation [^{3}H]SR144528.

5 mm ^{3}H probe with ^{1}H decoupling coil. Spectra were obtained as a 16 K free induction decay over a spectral width or 5550 Hz. A ^{1}H-coupled ^{3}H spectrum was acquired in 4000 transients with a 1 s interpulse delay using a pulse sequence that gated the ^{1}H decoupler off during acquisition. A ^{1}H-decoupled ^{3}H spectrum was acquired in 10240 transients with a 1 s interpulse delay. Both spectra were zero filled to 32 K prior to multiplication by a 0.1 Hz Lorentzian function and fourier transformation.

Ethyl 4-(4-chloro-3-methylphenyl)-2,4-dioxobutanoate (**2**)

Potassium *t*-butoxide (5.0 ml, 5.0 mmol, 1.0 M in THF) and anhydrous ethyl ether (150 ml) under dry nitrogen at −78°C was treated with lithium diisopropylamide (25.2 ml, 50.4 mmol, 2.0 M in heptane:THF:ethyl-benzene). After 15 min, 4-chloro-3-methylacetophenone **1** (8.50 g, 50.4 mmol) in 40 ml anhydrous ether was added dropwise with stirring over 25 min. After stirring for 30 min, the resulting orange-yellow mixture was treated with diethyl oxalate (6.85 ml, 50.4 mmol) and allowed to warm to ambient temperature. After the resulting orange solution was allowed to stand for 3 days it was extracted with 1 N HCl (3×200 ml) and the ether layer was dried over Na_2SO_4. The volatiles were removed *in vacuo* and subsequent high vacuum afforded 14.2 g of an orange oil. Chromatography on silica gel (10:1) eluting with 30% EtOAc:hexanes and combining fractions that were homogeneous by TLC (SiO_2, 30% EtOAc:hexanes, PMA/Ce^{+4}) yielded 9.21 g (68%) of the title compound as an orange waxy solid. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 7.92 (dd, 1H, $J = 1.8$, 26.8 Hz, Ar-5-H), 7.76 (dd, 1 H, $J = 2.2$, 8.4 Hz, Ar-2-H), 7.42 (dd, 1H, $J = 8.2$, 25.7 Hz, Ar-6-H), 7.03 (s, 1H, C3-H of enolate), 4.41 (q, 2H, $J = 7.14$ Hz, **CH_2**-CH_3), 2.46 (s, 3H, Ar-CH_3), 1.42 (t, 3H, $J = 7.14$ Hz, CH_2-**CH_3**). MS $C_{13}H_{13}ClO_4$ 268 (M^+), 195, 153, 125, 84, 69.

5-(4-Chloro-3-methylphenyl)-1-H-pyrazole-3-carboxylic Acid (**3**)

Ethyl 4-(4-chloro-3-methylphenyl)-2,4-dioxobutanoate **2** (4.0 g, 14.9 mmol) was added to hydrazine monohydrate (0.746 g, 14.9 mmol) dissolved in 75 ml absolute ethanol and heated at reflux under nitrogen with stirring for 1.5 h. Solid NaOH (1.79 g, 44.8 mmol) was added to the solution affording a turbid mixture that was heated at reflux a further 1.5 h. The volatiles were removed *in vacuo* to afford an orange-white solid which was suspended in water and washed with ether (4×100 ml). The aqueous suspension was acidified with HCl to pH 2 to afford a yellow-white precipitate which was extracted with ether (6×100 ml), combined and dried over Na_2SO_4 to give 3.18 g (91%) of the title compound after filtration and removal of volatiles. This material was used in the next step without further purification. ^{1}H-NMR (250 MHz, $CDCl_3$/DMSO-d_6) δ 7.75 (br d, 1H, $J = 31$ Hz, Ar-5-H), 7.56 (d, 1H, $J = 8.4$ Hz, Ar-2-H),

7.31 (dd, 1H, $J = 8.0$, 21 Hz, Ar-6-H), 7.04 (s, 1H, pyrazole 4-H), 2.41 (s, 3H, Ar-Me).

N-[(1S)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-H-pyrazole-3-carboxamide (**4**)

5-(4-Chloro-3-methylphenyl)-1-H-pyrazole-3-carboxylic acid **3** (3.18 g, 13.5 mmol) was suspended in 200 ml dry toluene under dry nitrogen and treated dropwise with distilled thionyl chloride (3.21 g, 27.0 mmol) with stirring at ambient temperature. The reaction was heated at reflux for 1.25 h and the volatiles removed *in vacuo* to afford 3.15 g (92%) of the acid chloride as a yellow solid which was used directly in the next step.

The acid chloride (3.15 g, 12.4 mmol) suspended in 400 ml dry CH_2Cl_2 was treated with (-)-fenchylamine[7,9] (2.85 g, 18.6 mmol) in 5 ml dry CH_2Cl_2 followed by triethylamine (3.76 g, 37.2 mmol) added dropwise over 2 min with stirring under a nitrogen atmosphere. At 1 h TLC (SiO_2, 25% EtOAc:hexanes, UV, phosphomolybdic acid/ceric sulfate detection) showed the reaction to be complete. The reaction was extracted with saturated aqueous Na_2CO_3 (2×200 ml), 1 N HCl (2×200 ml), and saturated aqueous Na_2CO_3 (2×200 ml). Drying over Na_2SO_4, filtration and evaporation *in vacuo* yielded 3.48 g (76%) of the title compound as a tan solid that was 97% pure by HPLC (Waters C18, 75% CH_3CN:H_2O, 280 nm detection). ^{1}H-NMR (250 MHz, MeOD) δ 7.71 (br d, 1H, $J = 21$ Hz, Ar-5-H), 7.53 (d, 1H, $J = 8.2$ Hz, Ar-2-H), 7.40 (dd, 1H, $J = 8.2$, 14.0 Hz, Ar-6-H), 7.06 (br s, 1H, pyrazole 4-H), 3.78 (s, 1H, αH), 2.42 (s, 3H, Ar-Me), 1.80–1.23 (m, 7H, fenchyl CH_2 and CH), 1.16 (s, 3H, fenchyl Me), 1.09 (s, 3H, fenchyl Me′), 0.88 (s, 3H, fenchyl Me″).

[C^3H_2OH]-4-methylbenzyl Alcohol (**6**)

To $LiAlT_4$ (prepared from 0.15 mmol carrier free tritium gas, 0.24 mmol *n*-BuLi, 0.24 mol TMEDA, and 0.06 mmol $ALBr_3$ as reported[10]), an excess of methyl 4-methylbenzoate **5** in dry benzene (36 mg/ml) (0.5 ml, 0.12 mmol) was added and the reaction mixture was stirred for 1 h. Anhydrous ether (0.5 ml) was added affording a homogeneous solution and stirred for 15 min. The reaction was quenched with 0.1 ml water, 0.1 ml 15% NaOH, and 0.1 ml water and stored in 2 ml ethanol at -70°C overnight. Evaporation of the ethanol ($2 \times$) *in vacuo* without going to dryness and partitioning the concentrate between water and ether ($3 \times$) was followed by drying the combined organic layers over Na_2SO_4. This was concentrated and stored in toluene to afford 485 mCi (9%) of product that coelutes with authentic unlabeled 4-methylbenzyl alcohol on TLC radioscan (SiO_2, 25% ether-hexane). Significant product losses that were experienced were likely due to volatility and incomplete extraction that can be addressed by dilution of the quenched

reaction with ether and drying over Na_2SO_4 to eliminate ethanol evaporation and partitioning.

[C^3H_2Br]-4-methylbenzyl bromide (**7**)

Tritiated 4-methylbenzyl alcohol (389 mCi) **6** was freed from most of the solvent and transferred with two 0.25 ml dry CH_2Cl_2 washes, to CBr_4 (10 mg, 0.030 mmol) under dry nitrogen. A 1.24 M solution of triphenyl phosphine in dry CH_2Cl_2 (25 µl, 0.030 mmol) was added and stirred at ambient temperature for 1 h. Three further additions of CBr_4 (25 µl, 0.030 mmol) and triphenyl phosphine (25 µl, 0.030 mmol) in CH_2Cl_2 were required to consume all the starting 4-methylbenzyl alcohol (GC analysis; DB-17, 100°C, fid). This was due to consumption of reagent by residual ethanol and possibly TMEDA. The reaction mixture was chromatographed twice (once to remove problematic toluene which causes coelution of **7** and ester) on silica gel (0.27 g, 0.5 g in pentane) eluted with pentane. Concentrating the product containing fractions by bulb-to-bulb rotary evaporation at aspirator vacuum and ambient temperature afforded 141 mCi (36%) of radiochemically pure product (TLC-radioscan; SiO_2, hexane) that was free of ester (GC). The material was stored in 10 ml of pentane.

N-[(1S)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-([C^3H_2]-4-methylbenzyl)-pyrazole-3-carboxamide, tritium-labeled SR144528

Desbenzyl SR144528 **4** (3.16 mg, 0.0086 mmol) in 0.15 ml dry toluene and 60% NaH dispersion in mineral oil (1.23 mg, 0.0513 mmol) in 0.20 ml dry toluene was stirred in a capped 1 ml reacti-vial in a 65°C oil bath for 1 h under dry N_2. Tritium-labeled 4-methylbenzyl bromide **7** (141 mCi, 0.0039 mmol) was exchanged into ~0.1 ml dry toluene and transferred to the cooled sodium salt with two 75 µl toluene washes. The reaction was stirred in a 140°C oil bath for 20 h by which time only modest progress was observed by HPLC (Waters C-18, 8 mm × 10 cm × 10 µ Resolve RCM column, 85% CH_3CN:H_2O, UV, βRAM) and by TLC radioscan (SiO_2, pentane). A further addition of NaH (0.6 mg, 0.026 mmol) in 0.1 ml toluene was made and the reaction was heated an additional 10 h by which time the [^{3}H]-4-methylbenzyl bromide was now only ~11% of the total radioactivity (HPLC βRAM). Work up by quenching\partitioning between saturated brine and ether (3 ×), drying the combined organic layers over Na_2SO_4 with CH_2Cl_2 added, filtration and concentration under a stream of nitrogen afforded 68 mCi of predominantly target radioligand. Chromatography on a silica gel column (0.5 g in hexane) was conducted eluting with a step gradient of 3–10% EtOAc-hexane in 1 ml steps, followed by EtOAc elution while collecting 0.5 ml fractions. Fractions 15, 16 (each 92% by HPLC βRAM) and the EtOAc (74%) contained the

product with a close eluting shoulder (44.4 mCi total, 31%). The product containing fractions were chromatographed on the above HPLC analytical system in batches of up to 19 mCi with no loss of resolution to afford 20.3 mCi of the $\geqslant 99\%$ radiochemically pure (HPLC) title compound. A two-dimensional TLC-radioscan (SiO_2, 20% EtOAc-hexane), however, showed the presence of 16% of a slightly more polar radioactive impurity (or decomposition product). The product was then chromatographed on silica gel eluting with 10% EtOAc-hexane to afford 3.86 mCi of radiochemically pure product that was chromatographically identical to the unlabeled compound by HPLC and TLC-radioscan. The specific activity was determined by counting and UV quantitation ($\mathcal{E}_{239} = 24\,000\,l/mol\,cm$ in 85% CH_3CN:H_2O) to be 32.5 Ci/mmol. The material was stored in 85% CH_3CN-water mobile phase.

Acknowledgements

This work was supported by Federal funds from the National Institutes on Drug Abuse, National Institutes of Health, Department of Health and Human Services, under Contract No. N01DA-6-7054.

References

1. Barth F, Rinaldi-Carmona M, Millan J, Derocq J-M, Bouaboula M, Casellas P, Congy C, Oustric D, Sarran M, Calandra B, Portier M, Shire D, Breliere J-C, Le Fur G. In *Internation Cannabinoid Research Society*. ICRS: Stone Mountain, GA, 1997; 11.
2. Rinaldi-Carmona M, Barth F, Millan J, Derocq J-M, Casellas P, Congy C, Oustric D, Sarran M, Bouaboula M, Calandra B, Portier M, Shire D, Breliere J-C, Le Fur G. *J Pharmacol Exp Ther* 1998; **284**: 644–650.
3. Munro S, Thomas KL, Abu-Shaar M. *Nature (London)* 1993; **365**: 61–65.
4. Barth F, Casellas P, Millan J, Oustric D, Rinaldi M, Sarran M. Sanofi Recherche, 1997. patent no. W097/21682
5. Seltzman HH, Carroll FI, Burgess JP, Wyrick CD, Burch DF. *J Chem Soc, Chem Commun* 1995; **15**: 1549–1550.
6. Seltzman HH, Carroll FI, Burgess JP, Wyrick CD, Burch DF. *J Label Compd Radiopharm* 2002; **44**: 1–12.
7. Suchocki JA, May EL, Martin TJ, George C, Martin BR. *J Med Chem* 1991; **34**: 1003–1010.
8. Ingersoll AW, Brown JH, Kim CK, Beauchamp WD, Jennings G. *J Am Chem Soc* 1936; **58**: 1808–1811.
9. Ingersoll AW, DeWitt HD. *J Am Chem Soc* 1951; **73**: 3360–3362.
10. Andres H, Morimoto H, Williams PG. *J Chem Soc, Chem Commun* 1990; **8**: 627–628.
11. Kocienski PJ, Cernigliaro G, Feldstein G. *J Org Chem* 1977; **42**: 353–355.
12. Hooz J, Gilani SSH. *Can J Chem* 1968; **46**: 86–87.

