JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 851–859.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.724

Research Article

A facile and efficient synthesis of ^{14}C-labelled sulforaphane

Christopher A. D'Souza, Shantu Amin and Dhimant Desai*
Organic Synthesis Facility, Institute For Cancer Prevention, American Health Foundation Cancer Center, 1 Dana Road, Valhalla, NY 10595, USA

Summary

Isothiocyanates have gained considerable attention for their role as potent chemopreventive agents. Sulforaphane, **1a** (SFN), a naturally occurring isothiocyanate, was isotopically labelled in five steps starting from 3-(methylthio)-1-propanol (**2**). Reacting **2** with tosyl chloride in the presence of Et_3N yielded the tosylate **3**. Gently refluxing **3** with K^{14}CN in DMF gave the nitrile **4b**. Reduction to the amine **5b** was achieved using BH_3-THF. Oxidation with 30% hydrogen peroxide followed by treatment with thiophosgene yielded ($\pm$)[1-^{14}C]SFN, **1b**. The overall radiochemical yield was 4.4% based on the starting K^{14}CN.

Key Words: isothiocyanates; chemopreventive agents; sulforaphane; [^{14}C]potassium cyanide

Introduction

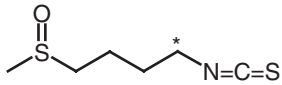
Sulforaphane, **1a** (SFN), a major isothiocyanate formed from the hydrolysis of glucosinolates (β-thioglucoside *N*-hydroxysulfates) by the enzyme myrosinase (thioglucoside glucohydrolase; EC 3.2.3.1), has attracted much attention for its induction of Phase II enzymes

*Correspondence to: D. Desai, Institute For Cancer Prevention, 1 Dana Road, Valhalla, NY 10595. E-mail: ddesai@ifcp.us

Contract/grant sponsor: NCI: contract/grant number: NCI-CB-77022-75 and N02-CN-95016
Contract/grant sponsor: Cancer Center Support grant; contract/grant number: P30 CA-17613

Received 23 January 2003
Revised 2 April 2003
Accepted 28 April 2003

[Glutathione transferases (GSTs) and NAD(P)H:quinone reductase (QR)].[1] Plants belonging to the family *Cruciferae*, particularly those of the genus *Brassica* (e.g. broccoli, Brussels sprouts, kale, cauliflower, turnips, cabbage)[2] are found to be rich in glucoraphanin (4-methylsulfinylbutyl glucosinolate). The hydrolysis of β-thioglucosides by myrosinase produces aglucons, which subsequently undergo non-enzymatic, intramolecular (Lossen) rearrangement to yield isothiocyanates, nitriles, thiocyanates, and epithionitriles.[3]



SFN

1a * = ^{12}C
1b * = ^{14}C

Epidemiological studies have shown that increased consumption of cruciferous vegetables is effective in decreasing the relative risk of cancer. Much of this chemopreventive effect has been attributed to the physiological effects of isothiocyanates (ITCs). Among the naturally occurring ITCs, SFN is the most potent inducer of Phase II proteins[1] and is found to activate Nuclear factor E2 p45-related factor 2 (Nrf2).[4] SFN has also been shown to induce cell cycle arrest and apoptosis in HT29 human colon cancer cells[5a] and human prostate cancer cells,[5b] and induce phase II enzymes in human prostatic cells.[5c] It was effective in reducing AOM-induced colonic aberrant crypt foci formation,[6a] mammary tumor multiplicity and incidence induced by DMBA,[6b] and DNA strand breaks induced by *N*-nitrosodimethylamine.[6c] More recently SFN has been shown to inhibit *Helicobacter pyroli* and prevent benzo[*a*]pyrene induced stomach tumors.[7]

Although a preliminary study has identified some major biliary and urinary metabolites in rats,[8] not much is currently known about the uptake or excretion, tissue distribution, and the identities of the metabolites of SFN in other species. [1-^{14}C]SFN will be useful to study its pharmacokinetics, tissue distribution, and metabolism.

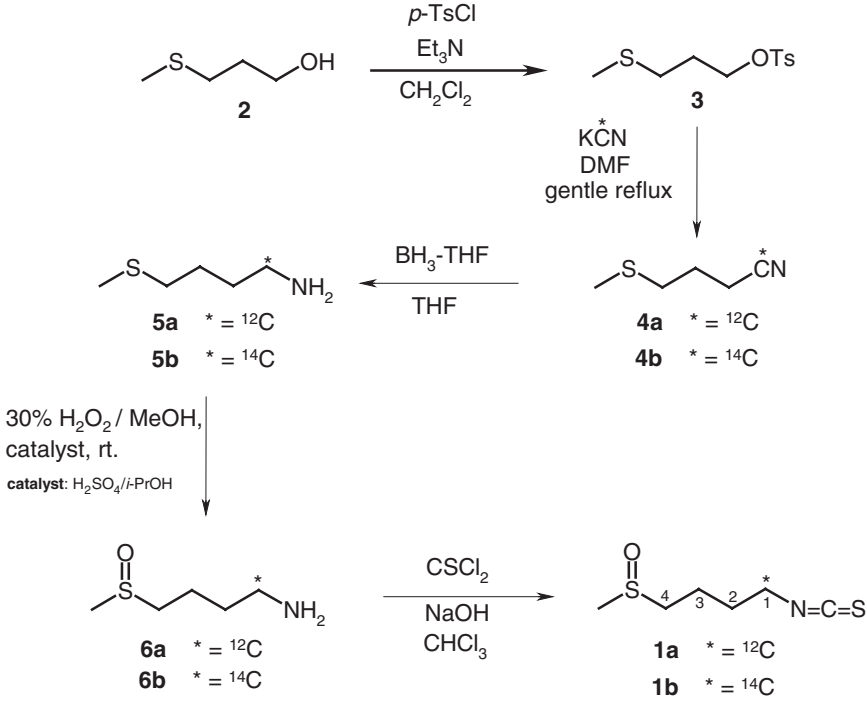
Results and discussion

Schmid and Karrer[9] were the first to synthesize **1a** starting from 4-bromobutyl-phthalimide, *via* a method that has been adopted by several groups[1a,10] with slight modifications. By reducing methyl 4-(methylthio)butyrate with $LiAlD_4$ and converting the resultant alcohol

to SFN, Kassahun *et al.*[8] achieved the synthesis of [1,1-$^2H_2$]SFN. In an attempt to synthesize **1a** in its naturally occurring configuration, Whitesell[11] and Schenk[12] made use of a chiral auxiliary, while Holland *et al.*[13] brought about the biotransformation of the prochiral sulfide (erucin) using *Helminthosporium* NRRL 4671. In a semi-synthetic way, glucoerucin, obtained from ripe seeds of *Eruca sativa*, was oxidized and subsequently hydrolysed by myrosinase to yield **1a**.[14] Although the yield was low, Kuhnert *et al.*[15] successfully synthesized [$^{14}CH_3$]SFN, using a slight modification of the Schmid and Karrer procedure.[9]

Two potential sites for carbon-14 substitution can be envisaged – the *S*-methyl group and the methylene group attached to the isothiocyanato functionality. We decided to label the latter, since this position is not involved in metabolic activation. The reaction conditions were optimized using non-radioactive material (Scheme 1).

Reacting 3-(methylthio)-1-propanol (**2**) with tosyl chloride in the presence of Et_3N yielded the tosylate **3** in good yield (81%). Gently refluxing **3** with KCN in DMF gave us nitrile **4a**. Reduction of **4a** to the



Scheme 1.

amine **5a** could be achieved using $LiAlH_4$. However, to avoid product isolation problems encountered during the work-up, we used BH_3THF.[16] Oxidation of the thioether to sulfoxide **6a** was achieved by employing the method of Mikolajczk *et al.*[17] [H_2O_2/MeOH/cat.H_2SO_4]. The amine sulfoxide **6a** was converted to the isothiocyanate **1a** using thiophosgene and NaOH in chloroform (yield 57% after column purification). Synthesis of [1-^{14}C]SFN, **1b**, started with tosylate **3** and [^{14}C]KCN in DMF. The sequence as depicted in Scheme 1 was followed and product purity was determined by TLC. Using this procedure, ($\pm$)[1-^{14}C]SFN (1.1 mCi) was synthesized with 96% radiochemical purity (HPLC data, Figure 1) and a specific activity of 54.2 mCi/mmol. The overall radiochemical yield was 4.4% based on the starting [^{14}C]KCN.

Experimental Section

General

ACS-grade solvents were used for the reactions. THF was distilled prior to use from a deep blue solution resulting from benzophenone and sodium. 3-Methylthio-1-propanol (**2**), *p*-toluenesulfonyl chloride, 30%

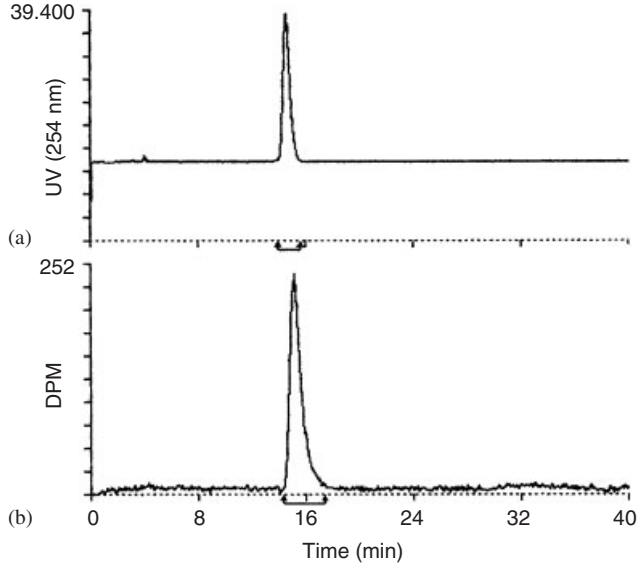


Figure 1. HPLC profile of [a] SFN and [b] [1-^{14}C]SFN

hydrogen peroxide, and BH_3THF were purchased from Aldrich Chemical Co. $K^{14}CN$ (specific activity = 54.2 mCi/mmol) was obtained from Moravek Biochemicals, Inc. (Brea, CA). Silica gel (60–200 mesh, J.T. Baker, Philipsburg, NJ) was used for column chromatography. TLC was performed with 0.2 mm pre-coated silica gel plates (60 F_{254}, EM Science, Gibbstown, NJ). Radiochemical purity was determined by scanning the TLC plates with a Bioscan System 200 imaging scanner (Bioscan Inc., Washington, DC) and also by autoradiography using X-ray films (Kodak), at room temperature for 2 h. All ^{1}H NMR spectra were recorded in $CDCl_3$ using a Bruker AM 360WB instrument. HPLC was carried out on a PRODIGY 5μ analytical ODS column (Phenomenex, 4.6 mm i.d. × 250 mm) with Waters 510 pumps equipped with a Gilson UV detector (254 nm) and β-RAM radioactivity detector (*Inus* Systems, Inc.). The radioactivity detector was operated in the homogeneous liquid scintillation mode upon addition of 2 ml/min of PICO FLUOR 40 (Packard) to the eluent passing through the UV detector. The eluant was 0.1% TFA in water/acetonitrile (80:20, by volume) at a flow rate of 1 ml/min at 25°C. GC-MS analyses were performed using a Hewlett-Packard 6890-5973 system, with HP-5MS column (30 m, 250 μm i.d., 0.25 μm film thickness, cross-linked to 5% phenyl methyl siloxane). Column oven temperature was initially set at 50°C for 4 min, then increased to 310 (ramp, 20°C/min) and held for 5 min. Helium was used as the carrier gas with a constant flow rate of 1.5 ml/min and 12.7 psi initial pressure. Mass spectra were obtained by electron ionization (EI) over a range of 20–300 atomic mass units. Ion source was 230°C, and the electron multiplier voltage was 2071 eV.

*3-(Methylthio)-1-(tosyloxy)propane [**3**]*

To a stirred solution of 3-methylthio-1-propanol (**2**) (1 ml, 9.7 mmol) and Et_3N (3 ml, 21.5 mmol) in CH_2Cl_2 (10 ml), chilled in an ice-bath, was added dropwise (over 45 min) and under nitrogen, a solution of *p*-TsCl (2.78 g, 14.6 mmol) dissolved in CH_2Cl_2 (20 ml). On completion of the addition, the reaction mixture was stirred at room temperature for 4–6 h. Next, the reaction mixture was diluted with water, and the organic phase was washed with dilute HCl, $NaHCO_3$, and water, then dried over $MgSO_4$, concentrated, and chromatographed (SiO_2, 90:10 Hexane:CH_2Cl_2) to afford the tosylate **3** as a colorless oil: 2.05 gms (81%). ^{1}H NMR δ 7.79 (d, J = 8.5 Hz, 2 H), 7.35 (d, J = 8.5 Hz, 2 H),

4.14 (t, J = 6.1 Hz, 2 H, O-CH_2), 2.51 (t, J = 7.1 Hz, 2 H, S-CH_2), 2.04 (s, 3 H, S-CH_3), 1.89–1.97 (m, 2 H, C-CH_2-C). MS (EI) m/z (%): 260 (M^+, 21), 155 (15), 105 (22), 91 (61), 89 (20), 88 (100), 73 (60), 65 (30), 61 (52).

*4-(Methylthio)butylnitrile [**4a**]*

Tosylate **3** (50 mg, 0.19 mmol) and KCN (25 mg, 0.38 mmol) in DMF (2 ml) were gently warmed (55–60°C) overnight in an atmosphere of nitrogen. This resultant solution was then cooled to room temperature, diluted with water (2 ml) and the products were extracted with hexane (4 × 30 ml). TLC analysis (CH_2Cl_2) with UV and iodine visualization showed good conversion of the tosylate **3** (R_f = 0.48) to the nitrile **4a** (R_f = 0.46). The organic layer was dried over $MgSO_4$, and the solvent removed *in vacuo* to yield the nitrile **4a**, a pungent smelling oil: 17 mg (77%). ^{1}H NMR δ 2.63 (t, J = 6.9 Hz, 2 H, CN-CH_2), 2.51 (t, J = 7.1 Hz, 2 H, S-CH_2), 2.10 (s, 3 H, S-CH_3), 1.91–1.99 (m, 2 H, C-CH_2-C). MS (EI) m/z (%): 115 (M^+, 79), 75 (10), 74 (11), 68 (13), 62 (25), 61 (100), 54 (14), 47 (18), 41 (28).

*4-(Methylthio)-1-butylamine [**5a**]*

To a solution of the nitrile **4a** (17 mg, 0.15 mmol) in dry THF (2 ml) was added dropwise 1 M BH_3-THF solution (1 ml, 1 mmol) under nitrogen. The mixture was stirred overnight at room temperature and monitored periodically by TLC (CH_2Cl_2, iodine visualization, R_f = 0.46) for the disappearance of starting material. The mixture was cooled to 0°C, 2N HCl (2 ml) was added to destroy excess borane, and the THF was distilled off. The aqueous residue was basified with 2N NaOH (3 ml) and extracted with $CHCl_3$ (3 × 20 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated to afford the amine **5a** as an oil 11 mg (63%). ^{1}H NMR δ 2.69 (t, J = 6.9 Hz, 2 H, N-CH_2), 2.51 (t, J = 7.2 Hz, 2 H, S-CH_2), 2.10 (s, 3 H, S-CH_3), 1.52–1.66 (m, 4 H, C-CH_2-CH_2-C). MS (EI) m/z (%): 119 (M^+, 61), 104 (39), 87 (51), 72 (48), 61 (48), 55 (11), 45 (31), 43 (44), 30 (100).

*(±)-4-(Methylsulfinyl)-1-butylamine [**6a**]*

30% H_2O_2 (12 µl, 0.12 mmol) was added to a round-bottomed flask containing a stirred solution of amine **5a** (11 mg, 0.09 mmol), MeOH

(3 ml) and the catalyst (3 µl each of *i*–PrOH and concentrated H_2SO_4) at room temperature. Within 2 h TLC analysis (silica gel, CH_2Cl_2/MeOH/NH_4OH (5:5:1), ninhydrin reagent as the developer) showed complete oxidation of sulfide **5a** ($R_f = 0.37$) to sulfoxide **6a** ($R_f = 0.12$). Water (2 ml) was added to the reaction mixture and the aqueous phase was saturated with NaCl and extracted with $CHCl_3$ (4 × 20 ml). The $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to give the sulfoxide **6a**, 8 mg (64%). ^{1}H NMR δ 2.66–2.79 (m, 4 H, N-CH_2, SO-CH_2), 2.57 (s, 3 H, SO-CH_3), 1.79–1.87 (m, 2 H, C-CH_2-C), 1.63–1.70 (m, 2 H, C-CH_2-C),[1a] MS: *m*/*z* 136 (MH^+).

*(±)-4-(Methylsulfinyl)-1-(isothiocyanato)butane (SFN) [**1a**]*

$CSCl_2$ (7 µl, 0.09 mmol) and 1 N NaOH (0.1 ml) were added at room temperature to a solution of sulfoxide **6a** (8 mg, 0.06 mmol) in $CHCl_3$ (2 ml). After 35 min the reaction mixture was partitioned between $CHCl_3$ (10 ml) and H_2O (5 ml). The separated organic layer was dried over $MgSO_4$, concentrated *in vacuo*, and chromatographed (SiO_2, 9:1 CH_2Cl_2:MeOH) to afford 6 mg of isothiocyanate **1a** as an oil in 57% yield. ^{1}H NMR δ 3.60 (t, J = 6.1 Hz, 2 H, N-CH_2), 2.66–2.77 (m, 2 H, SO-CH_2), 2.59 (s, 3 H, SO-CH_3), 1.85–1.99 (m, 4 H, C-CH_2-CH_2-C).[1a,11] MS (EI) *m*/*z* (%): 160 (91), 114 (11), 85 (8), 72 (1 0 0), 64 (18), 55 (35), 45 (8), 41 (7), 39 (9). [MS (EI) *m*/*z* (%): 160 (64), 114 (9), 85 (5), 72 (1 0 0), 64 (13), 55 (42)].[18]

*± 4-(Methylsulfinyl)-1-(isothiocyanato)-[1-^{14}C]butane ([1-^{14}C]SFN) [**1b**]*

Tosylate **3** (51 mg, 0.197 mmol) was converted to [1-^{14}C]SFN using [^{14}C]KCN (31 mg, 0.461 mmol, 25 mCi) through a series of reactions as shown in Scheme 1. After each step, the pure unlabelled standard and the reaction products were allowed to migrate side by side on a TLC plate, followed by autoradiography. The unlabelled standard was visualized using iodine, ninhydrin, or UV. To minimize contamination and losses, the products were purified by column chromatography only after the last step. Using this procedure, [1-^{14}C]SFN was synthesized with 96% radiochemical purity (HPLC) and a specific activity of 54.2 mCi/mmol. The overall radiochemical yield was 4.4% based on the starting [^{14}C]KCN.

Conclusion

A facile and efficient route to synthesize [1-^{14}C]SFN has been developed. [1-^{14}C]SFN will be very useful to investigate its chemopreventive mechanisms. The methodology reported herein can be utilized with success to synthesize other natural and synthetic isothiocyanates like benzyl isothiocyanate (BITC), phenethyl isothiocyanate (PEITC), and phenylhexyl isothiocyanate (PHITC).

Acknowledgements

We thank Mr John Cunningham of our instrumentation facility, for his assistance with the GC-MS analyses and Ms. Ilse Hoffmann for editing this manuscript. This study was supported by NCI contract NCI-CB-77022-75, Cancer Center Support grant P30 CA-17613, and by NCI contract NO2-CN-95016.

References

1. (a) Zhang Y, Talalay P, Cho CG, Posner GH. *Proc Natl Acad Sci USA* 1992; **89**: 2399–2403. (b) Fahey JW, Talalay P. *Food Chem Toxicol* 1999; **37**: 973–979.
2. Rosa EAS, Heaney RK, Fenwic GR, Portas CAM. *Hortic Rev* 1997; **19**: 99–215.
3. (a) Van Etten CH, Daxenbichler J.E, Peters JE, Tookey HL. *J Agric Food Chem* 1966; **14**: 426–430. (b) Cole RA. *Phytochemistry* 1976; **15**: 759–762.
4. Thimmulappa RK, Mai, KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S. *Cancer Res* 2002; **62**: 5196–5203.
5. (a) Gamet-Payrastre L, Li P, Lumeau S, Cassar G, Dupont M-A, Chevolleau S. *Cancer Res* 2000; **60**: 1426–1433. (b) Chiao JW, Chung F-L, Kancherla R, Ahmed T, Mittelman A, Conaway CC. *Int J Oncol* 2002; **20**: 631–636. (c) Brooks JD, Paton VG, Vidanes G. *Cancer Epidemiol Biomark Prev* 2001; **10**: 949–954.
6. (a) Chung F-L, Conaway CC, Rao CV, Reddy BS. *Carcinogenesis* 2000; **21**: 2287–2291. (b) Zhang Y, Kensler TW, Cho CG, Posner GH, Talalay P. *Proc Natl Acad Sci USA* 1994; **91**: 3147–3150. (c) Barceló S, Macé K, Pfeifer AMA, Chipman JK. *Mutat Res* 1998; **402**: 111–120.
7. Fahey JW, Haristoy X, Dolan PM. *Proc Natl Acad Sci USA* 2002; **99**: 7610–7615.

8. Kassahun K, Davis M, Hu P, Martin B, Baillie, T. *Chem Res Toxicol* 1997; **10**: 1228–1233.
9. Karrer P, Schmid H. *Helv Chim Acta* 1948; **31**: 1497–1507.
10. Barcelo S, Gardiner JM, Gescher A, Chipman JK. *Carcinogenesis* 1996; **17**: 277–282.
11. Whitesell JK, Wong M-S. *J Org Chem* 1994; **59**: 597–601.
12. Schenk WA, Dürr M. *Chem Eur J* 1997; **3**: 713–716.
13. Holland HL, Brown FM, Larsen BG. *Tetrahedron: Asymmetry* 1994; **5**: 1129–1130.
14. Iori R, Bernardi R, Gueyrard D, Rollin P, Palmieri S. *Bioorg Med Chem Lett* 1999; **9**: 1047–1048.
15. Kuhnert N, Holst B, Williamson G. *J Label Compd Radiopharm* 2001; **44**: 347–354.
16. Vergne F, Aitken D, Husson H-P. *J Org Chem* 1992; **57**: 6071–6075.
17. Mikolajczyk M, Drabowicz J, Lyzwa P, Popielarczyk M. *Synthesis* 1990; 937–938.
18. Kore AM, Spencer GF, Wallig MA. *J Agri Food Chem* 1993; **41**: 89–95.

