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Research Article

Synthesis of 1, 1′, 2, 2′, 3, 3′, 4, 4′ - Octadeutero-Sulforaphane

Nikolai Kuhnert* and Ye Lu
Department of Chemistry, School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH, UK

Summary

Sulforaphane (SFN), a naturally occurring isothiocyanate present in broccoli, shows strong evidence of anti-carcinogenic activity. The mechanism of action, absorption, distribution, metabolism and excretion of the compound is however still poorly understood and requires a stable isotope labelled version of the compound for further studies. The paper describes an optimized synthesis of octadeutero-SFN.

Key Words: deuterium; isothiocyanate; anti-cancer compound

Introduction

The inverse relationship between vegetable and fruit consumption and the incidence of various cancers in humans is well established.[1] It is not only the high concentration of vitamins, minerals and fibres that are responsible for these effects but plant derived compounds refered to as phytochemicals.[2]

One major subgroup of phytochemicals are the glucosinolates (GLS) e.g. Glucoraphanin **1**. GLS *per se* are biologically inactive but following cell disruption, the GLSs undergo enzymatic or non-enzymatic hydrolysis[3–5] to give as major products isothiocyanates (e.g. sulforaphane) to which the anticarcinogenic effects of broccoli-rich diets have been attributed.[6,7] As a result of a statement by the National Cancer Institute SFN is one of the 40 most promising anticarcinogens.[1] Based on epidemiological data[1,8] and experimental findings from numerous *in vivo* and *in vitro* studies on the biological activity of SFN,[9–11] there is a great interest in this particular isothiocyanate, which is found mainly in broccoli.[12,13]

*Correspondence to: N. Kuhnert, Department of Chemistry, School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH, UK. E-mail: n.kuhnert@surrey.ac.uk

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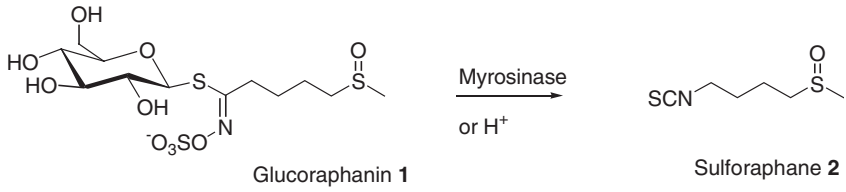


Glucoraphanin **1** Sulforaphane **2**

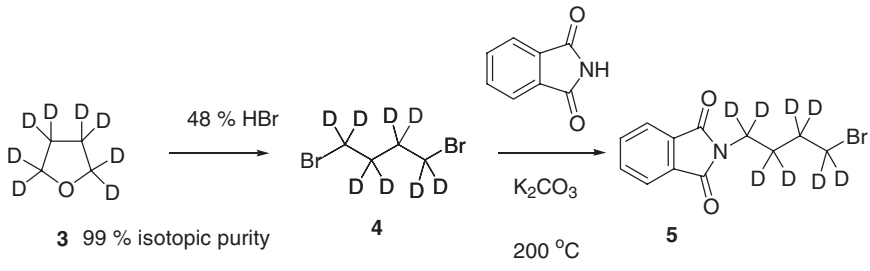
Using different models it has been shown that SFN is a very potent phase II enzyme inducer particularly of the glutathione *S*-transferase the principal enzyme system involved in the detoxification of carcinogenic chemicals. It has also been shown to suppress the activity of cytochrome P-450 enzymes involved in the bioactivation of chemical carcinogens. In this way it alters the balance of phase I/phase II enzyme metabolism in such a way that the activation of carcinogens during phase I is decreased and the rate of detoxification is increased. That is why SFN may play an important role in cancer prevention.[14–16] Recent results show that SFN is also able to induce cell cycle arrest and apoptosis in HT29 human colon cancer cells[17] and induce an anti-inflammatory response.[18] Thus SFN belongs to a small group of compounds enabling dual inhibitory action in carcinogenesis. However, the mechanisms of absorption, bioavailibility and metabolism are poorly understood. In order to address these questions related to bioavailability and mechanism of biological effects there is a need for stable isotope labelled SFN, which will enable us to

(1) Identify possible metabolites and conjugates with biological (macro) molecules e.g. enzymes, peptides, amino acids.

(2) Obtain pharmacokinetic data on absorption and distribution.

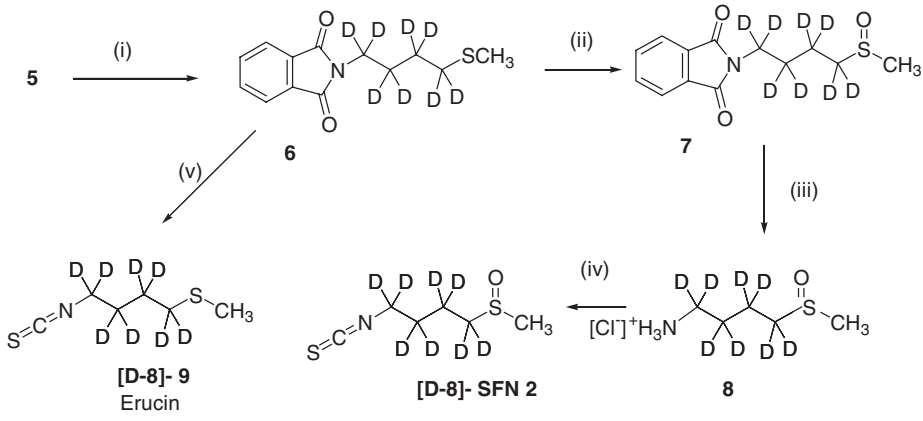
Results and discussion

Four chemical syntheses of sulforaphane **2** have been reported to date.[19–22] Our synthetic strategy for the synthesis of a stable isotope labelled **2** follows our published procedure concerned with the synthesis of radiolabelled SFN.[21] We reasoned that for the required mass spectrometric analysis of SFN and metabolites a high number of deuterium labels would be advantageous. Furthermore, the labels should be placed in positions that are not prone to H/D exchange. Hence, we decided to place the deuterium labels in the four carbon chain separating the NCS and methylsulfinyl moieties. We started our synthesis with d_8-tetrahydrofuran as a commercially available precursor. Using standard conditions we transformed d_8-THF **3** into d_8-1,4 dibromobutane **4**. Standard Gabriel procedure afforded the d_8-*N*-(4-bromo-butyl) phthalimide **5** in good yield without considerable H/D exchange, despite the strongly basic conditions employed.



Nucleophilic substitution of bromide **5** with NaSMe afforded the d_8-thioether **6** in good yield and excellent isotopic purity. Oxidation of **6** using sodium periodate in MeOH was shown to be the condition of choice to yield the sulfoxide **7** in almost quantitative yield. Cleavage of the phthalimide using hydrazine was uneventful to give the amine **8**, which was isolated as its hydrochloride.

For the final transformation to give sulforaphane thiophosgene and 3 equivalents of NaOH in a diethylether/water two phase solvent system gave the title compound in good overall chemical and labelled yield.



Reagents and conditions: (i) NaSMe/EtOH; (ii) $NaIO_4$/MeOH; (iii) 1. H_4N_2/EtOH, 2. HCl; (iv) $Cl_2C=S$, NaOH; (v) 1. H_4N_2/EtOH, 2. HCl, 3. $Cl_2C=S$, NaOH.

Yields and analytical data are summarized in Table 1. With the exception of **5** all unlabelled compounds have been described in the literature.[19,20] Both **4**[23] and **5**[20] have been prepared according to literature procedures. We report the analytical data of the labelled materials. All stable isotope labelled compounds displayed a characteristic C–D stretching frequency in the IR spectra and quintets in the ^{13}C-NMR spectra for the corresponding CD_2 carbons in the positions expected.

The deuterium incorporation and extent of labelling was confirmed with a variety of analytical methods. Firstly mass spectra did give the total deuterium

Table 1. Yield and spectroscopic data of compounds 4–9

Compound	Yield (%)	IR (cm^{-1})[a]	^{13}C-NMR[b]	^{1}H-NMR[c]	^{2}H-NMR[d,e]
4	82	2164, 2123, 2050 (C–D)	31.7 (quin, $J = 22.9$ Hz), 29.9 (quin, $J = 19.6$ Hz)	–	–
5	61	2926 (C–H), 2126 (C–D), 1698 (C = O)	168.6, 134.2, 132.7, 123.4, 53.2 (quin, J 22.5 Hz), 36.2, 32.9 (quin, $J = 21.3$ Hz), 29.3 (quin, $J = 21.7$ Hz), 25.8 (quin, $J = 20.3$ Hz)	7.81 (dd, $J = 3$ Hz; 8.3 Hz, 2 H, Ar), 7.66 (dd, $J = 3$, 8.3 Hz, 2 H, Ar)	3.69 (br, CD_2N), 3.41 (br, CD_2Br), 1.70–1.83 (br, CD_2 CD_2).
6	96	2925 (C–H), 2126 (C–D), 1698 (C = O)	168.9, 134.9, 132.0, 123.3, 53.2 (quin, J 22.5 Hz), 34.3 (quin, $J = 22.1$ Hz), 32.5, 26.0 (quin, $J = 21.7$ Hz), 18.3 (quin, $J = 20.3$ Hz)	7.81 (dd, $J = 3$; 8.3 Hz, 2 H, Ar), 7.66 (dd, $J = 3$, 8.3 Hz, 2 H, Ar), 2.04 (s, 3 H, SMe)	–
7	92	2126 (C–D)	165.6 (C = O), 134.2, 132.1, 123.5 (Ar), 53.9, 39.7, 37.2, 27.6, 23.1. 53.6(quin, J 22.4 Hz), 39.6 (quin, $J = 22.1$ Hz), 37.2, 27.2 (quin, $J = 21.7$ Hz), 18.3 (quin, $J = 20.3$ Hz)	7.80 (dd, $J = 3$; 8.3 Hz, 2 H, Ar), 7.68 (dd, $J = 3$, 8.3 Hz, 2 H, Ar), 2.57 (s, 3 H, SOMe)	3.71 (br, CD_2N), 2.99 (br, CD_2SO), 1.70–1.83 (br, CD_2 CD_2).
8	98	2126 (C–D)	53.2 (quin, $J = 22.5$ Hz), 34.3 (quin, $J = 22.1$ Hz), 32.5, 26.0 (quin, $J = 21.7$ Hz), 18.3 (quin, $J = 20.3$ Hz)	2.70 (s, 3 H, CH_3SO)	3.12 (br, CD_2N), 2.94 (br, CD_2SOMe), 2.72 (1.84 (br, 4 H, CD_2CD_2).
2	46	2126 (C–D), 2104 (NCS), 1039 (S = O)	Not available due to lack of solubility	2.70 (s, 3 H, CH_3SO)	3.12 (br, CD_2NCS), 2.94 (br, CD_2SOMe), 1.84 (br, CD_2CD_2).
9	50	2102 (NCS), 2126 (C–D)	44.9 (quin, $J = 22$ Hz), 33.3 (quin, $J = 22.4$ Hz), 28.9 (s), 26.0 (quin, $J = 21.7$ Hz), 15.6 (quin, $J = 20.3$ Hz)	2.06 (s, Me)	3.50 (br, CH_2NCS), 2.50 (br, CH_2S, 1.74, br, CH_2CH_2)

[a] Nujol mull.
[b] In $CDCl_3$ at 75 MHz.
[c] In $CDCl_3$ at 300 MHz.
[d] In $CHCl_3$/ $CDCl_3$ at 76.7 MHz.
[e] In D_2O for compound 8.

Scheme 1. Fragmentation of deuterated compounds 4–9

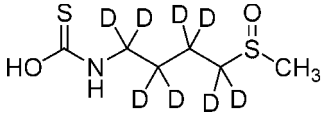
Table 2. Mass spectral data and isotopic purity

Compound	Mass	(m/z)[a]	Total % deuteration[b]	Deuteration at C-1 (%)[c]	Deuteration at C-4 (%)[c]
4	$C_4D_8Br_2$	222, 224, 226	> 99	99	99
5	$C_{10}H_4D_8BrNO_2$	265, 267	> 99	99	99
6	$C_{11}H_7D_8NO_2S$	233	97	96	97
7	$C_{11}H_7D_8NO_3S$	249[d]	95	94	95
8	$C_5H_5D_8NOS$	144	95	94	96
2	$C_6H_3D_8NOS_2$	185	92	90	93
9	$C_6H_3D_8NOS_2$	169	94	93	95

[a] All mass spectra were obtained using CI and isobutane as the ionizing gas.
[b] d_8 - is defined as 100% and d_0 as 0% deuteration.
[c] As estimated from the fragment ions A and B.
[d] Molecular ion $[M+H]^+$.

content of each individual compound. A direct comparison with a simulated mass spectrum and use of the established software (Thermo-Finnigan standard software was used.) did allow estimation of the total deuterium incorporation. Analysis of the fragment ions as well as integration of signals in the ^1H NMR spectra at 500 MHz enabled the determination of the deuterium incorporation at the C_1 and C_4 sites. The typical fragment ions allowing this estimation arise from a cleavage α to the nitrogen functionality resulting in an R_2NCD_2 fragment **A** and a second molecular ion **B** (Scheme 1). The results are summarized in Table 2.

As expected a small degree of H/D exchange is taking place at the most acidic site α to the methylsulfinyl moiety. H/D exchange is however not observed at the other sites. H/D exchange analysis of d_8-1 in water at pH 7 over a period of 4 days using GC-MS did reveal a 65% H/D exchange at C-1 along with a conversion of the NCS functionality into its hydrated derivative. The structure of the hydrated derivative is as follows:



m/z 202

Analytically pure d_8-erucin could be obtained by a similar method omitting the oxidation step. Erucin is the major isothiocyanate found in rocket salad and shares the same biological activity as sulforaphane.

Conclusion

We have established a reliable and efficient synthesis of octadeutero-sulforaphane and erucin. We will use these stable isotope labelled compounds in biological studies to find answers to problems relating to their metabolism and pharmocokinetics so as to ultimately be in a position to issue sound advice to the consumer regarding *brassica* consumption and their potential health benefits.

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