JOC Article
pubs.acs.org/joc

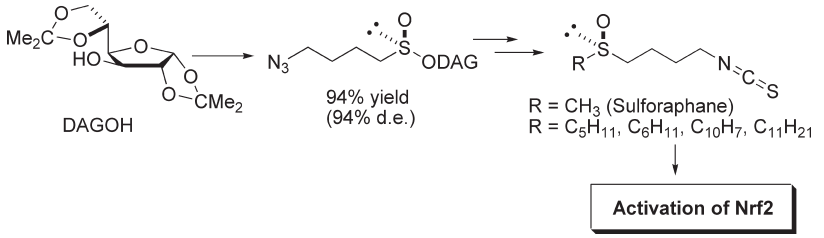
Enantiopure Sulforaphane Analogues with Various Substituents at the Sulfinyl Sulfur: Asymmetric Synthesis and Biological Activities

Noureddine Khiar,*[,†] Sabine Werner,[§] Siham Mallouk,[†] Franziska Lieder,[§] Ana Alcudia,[‡] and Inmaculada Fernández*[,‡]

[†]*Instituto de Investigaciones Químicas, C.S.I.C-Universidad de Sevilla, c/. Américo Vespucio, 49., Isla de la Cartuja, 41092 Sevilla, Spain,* [‡]*The Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain, and* [§]*Department of Biology, Institute of Cell Biology, ETH Zurich, Zurich, Switzerland*

khiar@iiq.csic.es; inmaff@us.es

Received April 15, 2009

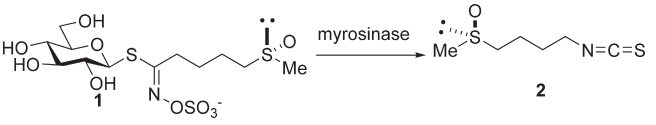


A convergent and high-yielding approach for the asymmetric synthesis of sulforaphane **2** and four analogues differently substituted at the sulfinyl sulfur has been developed. The key step of the synthesis is the diastereoselective synthesis of sulfinate ester **23**-S_S, using the DAG (diacetone-D-glucofuranose)-methodology. The biological activity of these compounds as inductors of phase II detoxifying enzyme has been studied by determining their ability to activate the cytoprotective transcription factor Nrf2.

Introduction

Compelling evidence from epidemiological and clinical studies has established that populations with a rich diet of broccoli and other cruciferous vegetables such as cauliflower, watercress, Brussels sprouts, and cabbage are less prone to develop certain types of cancer.[1] The chemical carcinogenesis prevention and chemotherapeutic benefit effects have been attributed to the high content of phytochemicals containing an isothiocyanate functional group.[2] These organic isothiocyanates are not produced as such by the plants, but they rather result from the enzymatic action of myrosinase (a thioglycosidase) on natural glucosinolates (β-thioglucoside-*N*-hydroxysulfates) such as glucopharin **1**, Scheme 1.[3] Myrosinase EC3.2.3.1. catalyzes the hydrolysis of the thioglycosidic bond leading to unstable intermediates, which at physiological pH, predominantly undergo

SCHEME 1. Biosynthetic Pathway to (R_S)-Sulforaphane 2: Myrosinase Deglycosylation of Glycopharin 1 Followed by Lossen Rearrangement



Lossen rearrangements to the corresponding isothiocyanate, such as **2**.[4] (R_S)-1-Isocyanato-4-(methylsulfinyl)butane (sulforaphane **2**), first isolated in 1992 from broccoli extracts,[5] is the main inducer of phase II detoxifying enzymes and is well-documented as a powerful chemopreventive agent.[6] In this regard, animal studies on rats have established the chemopreventive activity of sulforaphane against colon cancer[7] and recent preclinical and clinical studies have

*To whom correspondence should be addressed. N.K.: phone + 34 954489559; fax +34 954460565. I.F.: phone +34 954556738.

(1) (a) Beecher, C. W. *Am. J. Clin. Nutr.* **1994**, *54*, 1166S. (b) Lampe, J. W.; Peterson, S. *J. Nutr.* **2002**, *132*, 2991.

(2) (a) Conaway, C. C.; Yang, Y. M. *Curr. Drug Metab.* **2002**, *3*, 233. (b) Fenwick, G. R.; Heaney, R. K. *Food Chem.* **1983**, *11*, 249.

(3) (a) Bone, A. M.; Rossiter, J. T. *Physiol. Plant.* **1996**, *97*, 194. (b) Wittstock, U.; Halkier, B. A. *Trends Plant Sci.* **2002**, *7*, 263.

(4) Gil, V.; Macleod, A. J. *Phytochemistry* **1980**, *19*, 2547. (b) Halkier, B. A.; Gershenzon, J. *Annu. Rev. Plant Biol.* **2006**, *57*, 303.

(5) Zhang, Y.; Talalay, P.; Cho, C.-G.; Posner, G. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399.

(6) Juge, N.; Mithen, R. F.; Traka, M. *Cell. Mol. Life Sci.* **2007**, *64*, 1105.

(7) Chung, F.-L.; Conaway, C. C.; Rao, C. V.; Reddy, B. S. *Carcinogenesis* **2000**, *21*, 2287.

Published on Web 07/13/2009 DOI: 10.1021/jo9007749

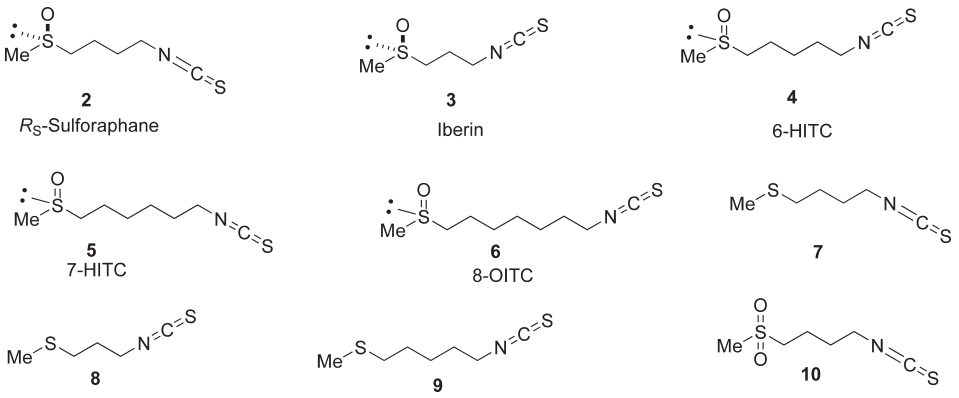


FIGURE 1. Structure of sulforaphane and some analogues.

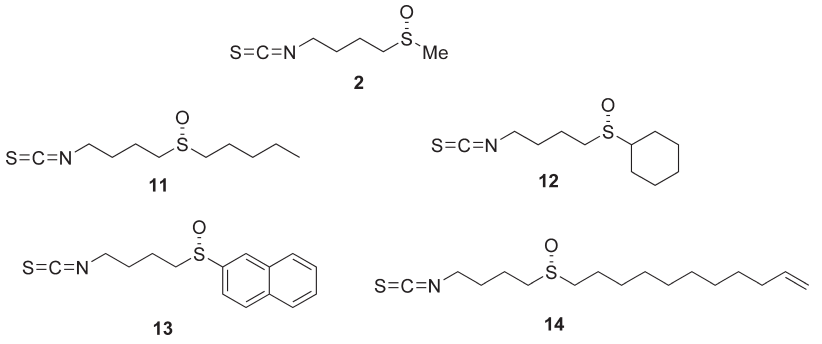


FIGURE 2. Structure of sulforaphane analogues synthesized in this work.

confirmed the chemopreventive activity of sulforaphane in women at risk for breast cancer.[8]

The structural characteristics of isothiocyanate seem to play a key role in their biological activities. It has been shown that small changes in the isocyanate structure induce an important effect on the chemopreventive activities.[9] In nature, besides the common glycone moiety, the glycosinolate molecules possess a variable side chain derived from amino acids.[10] Although a large number of natural and synthetic analogues have been described, up to now none of them surpass the activity of natural sulforaphane.[9,11] In this regard, a key characteristic of the sulforaphane moiety is the presence in the side chain of a methylsulfinyl group. The presence of the sulfinyl group is not limited to sulforaphane but has been described for other isothiocyanates such as iberin **3**,[12] 6-methylsulfinylhexyl isocyanate **4** (6-HITC),[13] 7-methylsulfinylheptyl isocyanate **5** (7-HITC), and 8-methylsulfinyloctylisocyanate **6** (8-OITC)[14] (Figure 1).

With regard to the importance of the oxidation state of the sulfur atom on the biological activity, it is important to indicate that both the natural thioethers **7**−**9**, and the sulfone **10**, Figure 1, are less active than the corresponding sulfoxide derivatives.[5,6,14] Even though two enantiomers are possible, the natural sulforaphane and iberin exist as a single enantiomer with an R_S absolute configuration.[15] On the other hand, while the absolute configurations of methyl sulfoxides **4**−**6** have not been determined yet, they have most probably the R_S absolute configuration at sulfur, as the oxidation of the sulfide in the gluosinolates by flavin monooxygenase has been recently reported to be stereospecific.[16] Nevertheless, up to now only one study has addressed the sulfur chirality and the antitumoral activity of sulforaphane.[17] Additionally, while the influence of the chain length linking the electrophilic isothiocyanate group and the Lewis basic sulfinyl moiety on their anticancer activity has been determined, as far as we know there is no study on the influence of the substituent at the sulfinyl sulfur.

To address this problem, in the present study we have developed a convergent and high-yielding approach to optically pure sulforaphane analogues **11**−**13**, Figure 2, with different substituents at the sulfinyl sulfur and we have compared their activities with those of the parent sulforaphane **2**. The biological activity of these compounds as inductors of phase II detoxifying enzyme has been studied by determining their ability to activate the cytoprotective transcription factor Nrf2.

(8) Cormblatt, B. S.; Ye, L.; Dikova-Kostova, A. T.; Erb, M.; Fahey, J. W.; Singh, N. K.; Chen, M.-S. A.; Stierer, T.; Garret-Mayer, E.; Argani, P.; Davidson, N. E.; Talalay, P.; Kensler, T. W.; Visvanathan, K. *Carcinogenesis* **2007**, *28*, 1485.

(9) (a) Zhang, Y.; Kensler, T. W.; Cho, C.-G.; Posner, G. H.; Talalay, P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3147. (b) Posner, G. H.; Cho, C.-G.; Green, J. V.; Zhang, Y.; Talalay, P. *J. Med. Chem.* **1994**, *37*, 170. (c) Mays, J. R.; Weller Roska, R. L.; Sarfaraz, S.; Mukhtar, H.; Rajski, S. C. *ChemBioChem* **2008**, *9*, 729.

(10) Halkier, B. A.; Gershenzon, J. *Ann. Rev. Plant Biol.* **2006**, *57*, 303.

(11) Fahey, J.; Zalckmann, A. T.; Talalay, P. *Phytochemistry* **2001**, *56*, 5.

(12) Kore, A. M.; Jeffery, E. H.; Wallig, M. A. *Food Chem. Toxicol.* **1993**, *31*, 723.

(13) Morimitsu, Y.; Nakagawa, Y.; Hayashi, K.; Fujii, H.; Kumagai, T.; Nakaruma, Y.; Osawa, T.; Horio, F.; Itoh, K.; Iida, K.; Yamamoto, M.; Uchida, K. *J. Biol. Chem.* **2002**, *277*, 3456.

(14) Rose, P.; Faulkner, K.; Williamson, G.; Mithen, R. *Carcinogenesis* **2000**, *21*, 1983.

(15) Vergara, F.; Wenzler, M.; Hansen, B. G.; Kliebenstein, D. J.; Halkier, B. A.; Gershenzon, J.; Schneider, B. *Phytochemistry* **2008**, *69*, 2737.

(16) Hansen, B.; Kliebenstein, D. J.; Halkier, B. A. *Plant J.* **2007**, *50*, 902.

(17) Zhang, Y.; Tang, L. *Acta Pharmacol. Sin.* **2007**, *28*, 1343.

Results and Discussion

The past decade has witnessed an increasing interest toward the preparation of chiral sulfinyl derivatives in relation to their application in asymmetric synthesis.[18] This interest was mainly directed toward the preparation of chiral sulfoxides as a consequence of their high efficiency and wide applicability as chiral controllers in asymmetric carbon−carbon and carbon−heteroatom bonds formation. On the other hand, even though the sulfur chirality has not been as widely addressed as carbon chirality in natural products and medicinal chemistry,[19] there are a number of biologically active compounds which have in their structure a sulfinyl group and which exhibit differential stereochemically dependent metabolism and enzyme inhibition.[18a]

Although the natural sulforaphane exists as a single enantiomer in nature, most studies conducted on its biological activities have been conducted by using the racemic form. As an alkyl methyl sulfoxide, the synthesis of enantiopure sulforaphane is not straightforward, as the main approaches for the synthesis of enantiopure sulfoxides developed so far fail in the preparation of simple dialkyl sulfoxides. Actually, there is no asymmetric catalytic oxidation able to give enantiopure dialkyl sulfoxides from the corresponding prochiral thioethers.[20] In the particular case of sulforaphane, only three approaches have been reported so far, and two of them give the natural enantiomers in enantioenriched form. The first one, developed by Whitesell's group, used *trans*-2-phenylhexanol as a chiral auxiliary for the synthesis of diasteromerically pure methyl sulfinate ester **15**, from which R_S-sulforaphane was obtained through 5 steps and 42% overall yield.[21] Holland's group used a microbial oxidation with Helminthosporium *sp* of the prochiral sulfide **8**, which afforded R_S-sulforaphane in 86% ee and 54% yield.[22] Finally, Schenk's group employed a method based on a diastereoselective oxidation of cationic ruthenium complex **16**, which leads to R_S-sulforaphane with a modest 80% ee (Figure 3).[23]

Noteworthy, none of these methods is able to give the designed analogues **11**−**14**, as the substituent at sulfur is fixed to a methyl group. Therefore, to develop a convergent approach to these kinds of sulfoxides, the needed sulfinylating agent should be able to efficiently transfer the linear alkyl sulforaphane side chain in enantiomeric form. In this regard, the "DAG-methodology" developed in our group seems the method of choice for several reasons.[24] Among the available strategies in the literature, it is the only one able to give a diasteromerically pure sulfinate ester with a nonhindered alkyl chain at the sulfinyl sulfur. Additionally, based on our experimental and theoretical studies,[25] both epimers at sulfur

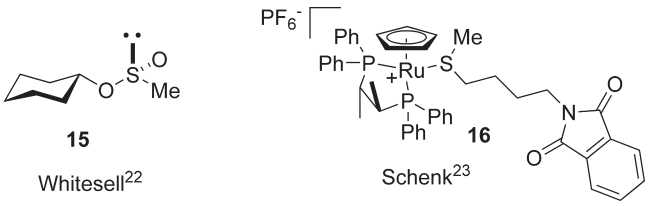
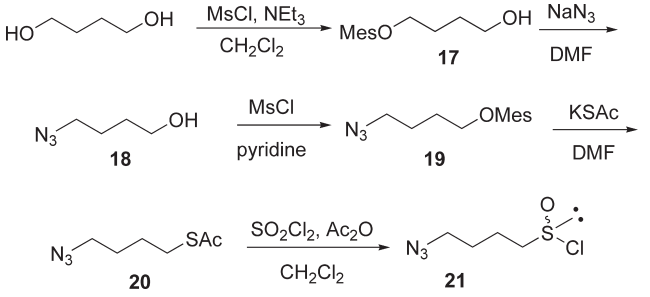
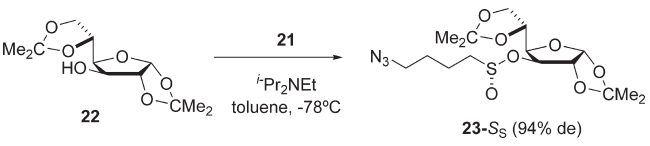


FIGURE 3. Diasteromeric intermediates used for the synthesis of enantiopure and enantioenriched R_S-sulforaphane.

SCHEME 2. Synthesis of 1-Azidobutanesulfinyl Chloride 21



SCHEME 3. Diasteroselective Synthesis of DAG Sulfinate Ester 23-S_S



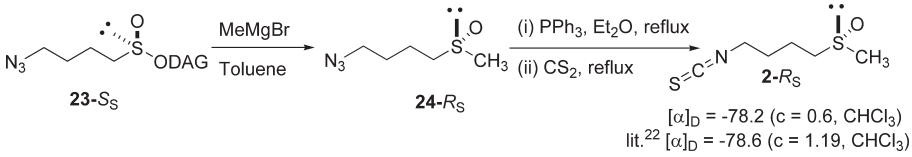
should be accessible in an enantiodivergent manner by a simple change of the base used to catalyze the reaction through a dynamic kinetic transformation of the starting sulfinyl chloride.[26] For the synthesis of desired 1-azidobutanesulfinyl chloride a de novo approach has been developed starting from butane diol (Scheme 2).

A statistical mesylation of 1,4-butanediol afforded the monomesylated compound **17**, which was transformed in 58% yield to the corresponding azide by treatment with sodium azide in DMF. A second mesylation of azido alcohol **18** led to the fully protected compound **19** in 56% yield, which after treatment with sodium thioacetate afforded compound **20** in 73% yield. The direct transformation of thioacetate **20** to the desired sulfinyl chloride **21** has been achieved by treatment with sulfuryl chloride and acetic anhydride in methylene chloride.[27]

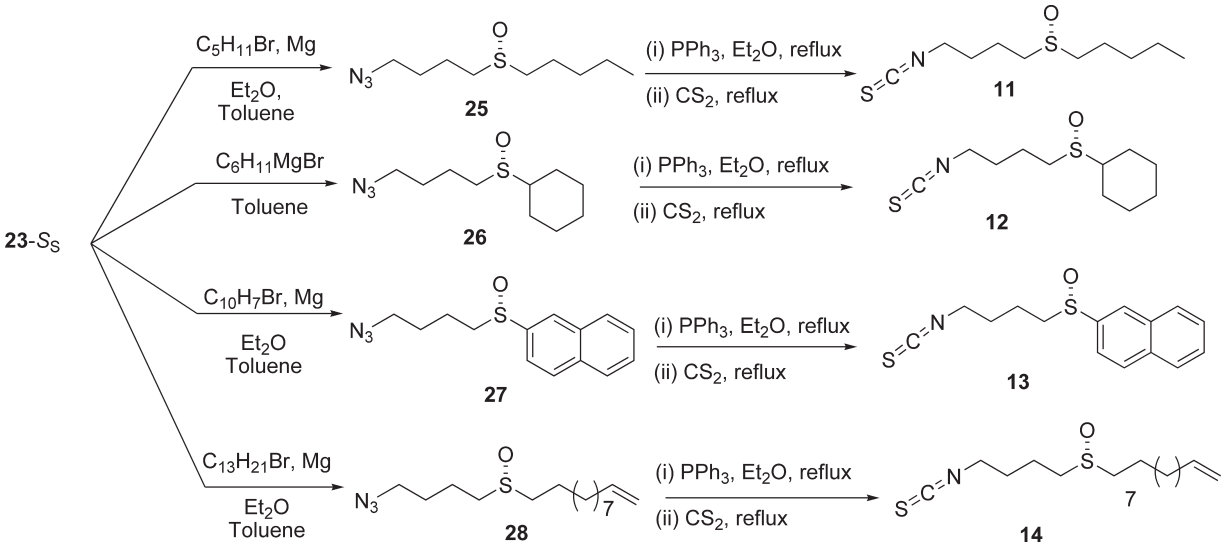
Taking into account that the reaction of sulfinate esters with Grignard reagents takes place with inversion of configuration at the sulfinyl sulfur, the S_S-DAG sulfinate ester is the desired intermediate for the synthesis of the natural sulforaphane. To do so, diacetone-D-glucose **22** was reacted with a freshly prepared sulfinyl chloride **21** by using Hunig's base as catalyst, which afforded the desired sulfinate ester **23**-S_S in 90% yield and in 94% diastereomeric excess (Scheme 3).

(18) (a) Fernández, I.; Khiar, N. *Chem. Rev.* **2003**, *103*, 3651. (b) Pellissier, H. *Tetrahedron* **2006**, *62*, 5559.
(19) Bentley, R. *Chem. Soc. Rev.* **2005**, *34*, 609.
(20) Legros, J.; Delhi, J. R.; Bolm, C. *Adv. Synth. Catal.* **2005**, *347*, 19.
(21) Whitesell, J. K.; Wong, M.-S. *J. Org. Chem.* **1994**, *59*, 597.
(22) Holland, H. L.; Brown, F. M.; Larsen, B.; Zabic, M. *Tetrahedron: Asymmetry* **1995**, *6*, 1569.
(23) Schenk, W. A.; Dürr, M. *Chem.—Eur. J.* **1997**, *3*, 713.
(24) (a) Fernández, I.; Khiar, N.; Llera, J. M.; Alcudia, F. *J. Org. Chem.* **1992**, *57*, 6789. (b) Khiar, N.; Fernández, I.; Alcudia, F. *Tetrahedron Lett.* **1994**, *35*, 5719. (c) El Ouazzani, H.; Khiar, N.; Fernández, I.; Alcudia, F. *J. Org. Chem.* **1997**, *62*, 287. (d) Fernández, I.; Valdivia, V.; Khiar, N. *J. Org. Chem.* **2008**, *73*, 745.
(25) (a) Balcells, D.; Maseras, F.; Khiar, N. *Org. Lett.* **2004**, *6*, 2197. (b) Balcells, D.; Ujaque, G.; Fernández, I.; Khiar, N.; Maseras, F. *J. Org. Chem.* **2006**, *71*, 6388. (c) Balcells, D.; Ujaque, G.; Fernández, I.; Khiar, N.; Maseras, F. *Adv. Synth. Catal.* **2007**, *349*, 2103.
(26) (a) Khiar, N.; Alcudia, F.; Espartero, J.-L.; Rodríguez, L.; Fernández, I. *J. Am. Chem. Soc.* **2000**, *122*, 7598. (b) Khiar, N.; Araújo, C. S.; Alcudia, F.; Fernández, I. *J. Org. Chem.* **2002**, *67*, 345.
(27) Lowik, D. W. P. M.; Liskamp, R. M. J. *Eur. J. Org. Chem.* **2000**, 1219.

SCHEME 4. Synthesis of Enantiopure Natural Sulforaphane 2-R_S



SCHEME 5. Synthesis of Enantiopure Sulforaphane Analogues 11−14



The diastereoselectivity of the reaction has been easily determined by ^{1}H NMR analysis of the crude. After screening various deuterated solvents, we have found that deuterated benzene is the best solvent for the determination of the stereochemical outcome of the reaction, by inducing a splitting of the anomeric protons, as well as that of the protons in the two and three position of diastereoisomers **23**-S_S and **23**-R.[28] With the sulfinylating agent **23**-S in hand, we first accomplished the synthesis of natural sulforaphane, to determine the scope of the reaction and to corroborate the absolute configuration of the sulfinate ester.

Condensation of methyl Grignard on the sulfinate ester **23**-S_S afforded 4-azidobutyl methyl sulfoxide **24**-R_S. Staudinger reaction of the azido derivative **24**-R_S with triphenylphosphine and subsequent aza Wittig-type condensation of the resulting iminophosphorane with carbon disulfide led to enantiopure natural sulforaphane **2**-R_S as shown by its physical properties (Scheme 4).

Once demonstrated, the absolute configuration of the intermediate sulfinate ester and its usefulness for the synthesis of natural sulforaphane was applied in the synthesis of the remaining analogues. Condensation of the adequate Grignard reagent on sulfinate ester **23**-S_S led to the desired azido sulfoxides **25**−**28**. A subsequent 2-reaction one-pot sequence leads to the enantiopure sulfinyl isocyantes **11**−**14** (Scheme 5).

Biological Activities of Sulforaphane Analogues. It has been shown that sulforaphane exerts potent anticancerogenic activity through the activation of the transcription factor NF-E2-related factor 2 (Nrf2).[29] Activated Nrf2 dimerizes with small Maf proteins or other leucine zipper proteins and binds as a heterodimer to the antioxidant response element (ARE) in the promoter of its target genes. Genes that are regulated by Nrf2 encode proteins that help to control the cellular redox status and protect the cell against oxidative damage. These include a series of enzymes that detoxify reactive oxygen species (ROS) and other antioxidant proteins, including NAD(P)H:quinone oxidoreductase (NQO1), several glutathione *S*-transferases (GST), γ-glutamylcysteine synthetase (γ-GCS), peroxiredoxin 1, and heme oxygenase 1 (HO-1).[30] With regard to the mechanism of activation of Nrf2 at the molecular level, it is widely accepted that the first step is the thiocarbamoylation of cysteines in the Nrf2 inhibitor protein Keap1 with the electrophilic isocyanate group of sulforaphane.[31] While it has been proposed initially that this activation is sufficient for the dissociation of the Nrf2-Keap1 complex,[32] recent in vivo studies have shown that this is not the case and reveal the existence of an alternative mechanism.[33] In any case, the key step of the process is the interaction of sulforaphane with Keap1, where the structure of sulforaphane must play a preponderant role. In this regard, the function of the sulfinyl group, which has not been determined yet, must be that of optimizing the

(28) For **23**-S_S, δ ppm: H-1 (5.92), H-2 (4.61), H-3 (4.94). For **23**-R_S, δ (ppm): H-1 (5.96), H-2 (5.04), H-3 (4.95). The diastereoselection can be confidently determined by integrating either the signals of the anomeric protons or the H-2 protons.

(29) Gao, X.; Talalay, P. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10446.
(30) Nguyen, T.; Sherratt, P. J.; Pickett, C. B. *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43*, 233.
(31) Dinkova-Kostova, A. T.; Massiah, M. A.; Bozak, R. E.; Hicks, R. J.; Talalay, P. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3404.
(32) Dinkova-Kostova, A. T.; Holtzclaw, W. D.; Cole, R. N.; Itoh, K.; Wakabayashi, N.; Katoh, Y.; Yamamoto, M.; Talalay, P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11908.
(33) Eggler, A. L.; Liu, G.; Pezzuto, J. M.; van Breemen, R. B.; Mesecar, A. D. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10070.

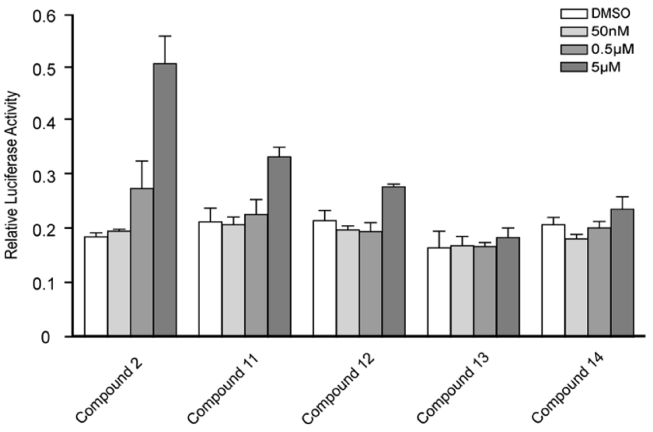


FIGURE 4. Dose-dependent effects of sulforaphane analogues on the activation of an ARE reporter gene. HaCaT keratinocytes were transiently transfected with the rNQO1-ARE luciferase reporter plasmid and the phRL-CMV *Renilla* luciferase vector. Transfected cells were incubated for 24 h with different concentrations of the sulforaphane **2** and analogues **11**−**14**. Results were normalized to the *renilla* luciferase activity. Values show mean ± SD from triplicate determinations. The result was reproduced in an independent transfection experiment.

side interaction of **2** with Keap1, probably through hydrogen bond interaction with amino acids near the reactive cysteines. This hypothesis is based on the fact that analogues with a hydrogen bond acceptor such a ketone group are as active as **2**, while those lacking this kind of group are less active.[9b] Thus, any change on the substituent at the sulfinyl group in **2** should have a substantial effect on the biological activity.

To determine the validity of this hypothesis, we investigated the capability of sulforaphane analogues **11**−**14** to activate Nrf2 by transfecting immortalized human keratinocytes (HaCaT cells) with a reporter construct containing a 31bp segment of the rat *Nqo1* promoter with the core ARE (pGL3-rNQO1 ARE).[34] The transfected cells were treated with five different concentrations of sulforaphane analogues (50 nM, 0.5 μM, 5 μM, 50 μM, and 500 μM), and the results are given in Figure 4. The concentrations 50 and 500 μM were toxic for the cells (data not shown). At lower concentrations, however, a clear difference between the original sulforaphane and its analogues was identified, with all analogues being much less active compared to the original compound. The activation of Nrf2 was intimately related to the nature of the substituent at the sulfinyl sulfur. As a general tendency, the sulforaphane analogues with an alkyl side chain were more active than the analogue with the aromatic sulfinyl group **13**. Within the dialkyl sulfoxides **11**, **12**, and **14**, the most active one is the pentyl sulfoxide **11**, while the dialkyl sulfoxide **14** with an extended alkylic chain is the least active. Taking all together, these results indicate that the activity of the sulforaphane analogues depends on the substituent of the sulfinyl sulfur, and that there is a close relation between their steric hindrance and their biological activity.

In conclusion, we have developed a new, convergent, and highly efficient approach for the synthesis of enantiopure sulforaphane and four of its analogues with either an aromatic or an alkylic substituent at the sulfinyl sulfur. The enantiopure sulfinyl thioureas were assayed in the activation of the cytoprotective transcription factor Nrf2. The results obtained indicate that there is a close relationship of Nrf2 activation and the steric demand of the substituent at the sulfinyl sulfur, which sheds some light on the rather unknown structural requirement for an effective interaction of sulforaphane and its biological inhibitor Keap1, and may help in the design of new and more effective sulforaphane analogues.

Experimental Section

General Methods. All reactions were run under an atmosphere of dry argon, using oven-dried glassware and freshly distilled dried solvents. THF and diethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane and toluene were distilled from calcium hydride. TLC was performed on Silica Gel GF254 with detection by charring with phosphomolybdic acid/EtOH. For flash chromatography, silica gel (230−400 mesh) was used. Columns were eluted with positive air pressure. Chromatographic eluents are given as volume to volume ratios (v/v). NMR spectra were recorded with (1H, 300 MHz) and (1H, 500 MHz) spectrometers. Chemical shifts are reported in ppm, and coupling constants are reported in Hz. Routine spectra were referenced to the residual proton or carbon signals of the solvent. The organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuum.

4-Hydroxybutyl methanesulfonate (17): To a solution of 1,4-butanediol (10 g, 9.86 mL, 110.9 mmol) and EtN_3 (12 g, 16.9 mL, 122 mmol) in CH_2Cl_2 (80 mL) at 0 °C was added dropwise methanesulfonyl chloride (12.7 g, 8.58 mL, 110.96 mmol). After 1 h at 0 °C, the reaction mixture was quenched with saturated NH_4Cl solution and extracted with CH_2Cl_2 twice, then the combined organic layers were dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel chromatography, using 20:1 CH_2Cl_2/MeOH, affording 5.07 g (27%) of **17** as a colorless liquid. R_f 0.40 (CH_2Cl_2/MeOH, 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.27 (t, 2H, J = 6.6 Hz), 3.78 (t, 2H, J = 6.6 Hz), 3.02 (s, 3H), 1.94−1.84 (m, 2H), 1.78−1.73 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 68.2, 62.4, 37.3, 27.4, 25.4.

4-Azidobutan-1-ol (18): To a solution of the monomesylate **17** (7 g, 41.58 mmol) in DMF (48 mL) was added sodium azide (10.82 g, 166.32 mmol). The reaction mixture was heated to 70 °C for 90 min, then cooled to room temperature and diluted with Et_2O (200 mL). The organic layer was washed with H_2O (140 mL) and brine (140 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give 2.8 g (58%) of **18** as a colorless oil. R_f 0.35 (EtOAc/hexanes, 2:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.70 (t, 2H, J = 6 Hz), 3.35 (t, 2H, J = 6.5 Hz), 1.72−1.67 (m, 4H), 1.54 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 62.2, 51.3, 29.8, 25.4.

4-Azidobutyl methanesulfonate (19): To a solution of 4-azido-1-butanol **18** (2.8 g, 24.33 mmol) in pyridine (17.5 mL) at 0 °C was added methanesulfonyl chloride (2.81 mL, 36.5 mmol) dropwise. After 5 h at room temperature, the reaction mixture was washed with H_2O and 10% HCl and extracted with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4, and evaporated. Flash chromatography, using 2:1 CH_2Cl_2/hexanes, gave **19** (2.5 g, 58%) as a colorless oil. R_f 0.54 (EtOAc/hexanes, 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.27 (t, 2H, J = 6.3 Hz), 3.36 (t, 2H, J = 6.6 Hz), 3.02 (s, 3H), 1.91−1.72 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 69.1, 50.7, 37.4, 26.4, 25.0.

4-Azidobutyl-1-thioacetate (20): To a solution of **19** (3.1 g, 17.62 mmol) in DMF (30 mL) was added potassium thioacetate

(34) Favreau, L. V.; Pickett, C. B. *J. Biol. Chem.* **1995**, *270*, 24468.

(2.4 g, 21.14 mmol) dropwise at room temperature. The reaction mixture was stirred overnight, washed with water, and extracted three times with EtOAc. The combined organics were washed with saturated $NaHCO_3$ solution and brine, filtered, and evaporated. The crude product was purified by silica gel chromatography, using 1:2 EtOAc/hexanes, affording 2.22 g (73%) of **20** as a colorless oil; R_f 0.65 (EtOAc/hexanes, 1:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.35−3.29 (m, 2H), 2.94−2.90 (m, 2H), 2.36 (s, 3H, CH_3), 1.71−1.66 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.8, 30.6, 28.4, 27.8, 26.8.

4-Azidobutane-1-sulfinyl chloride (21): To a solution of compound **20** (2.22 g, 12.83 mmol) in methylene chloride (42 mL) at −20 °C was added acetic anhydride (1.2 mL, 12.83 mmol) and sulfuryl chloride (2 mL, 25.66 mmol). The resulting mixture was stirred for 1 h at −5 °C and then the solvent was evaporated and the residue was dried under vacuum. The crude sulfinyl chloride, which was kept under argon, was used immediately for the preparation of sulfinate ester.

(*S*)-(1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl) 4-azidobutanesulfinate (23-S_S): To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (DAG) **22** (1.66 g, 6.41 mmol) and Hunig's base (4.4 mL) in anhydrous toluene, cooled to −78 °C and placed under argon atmosphere, was added 4-azidobutyl-1-sulfinyl chloride **21** (7.67 mmol) while the reaction mixture was being vigorously stirred. After the solution was stirred at −78 °C for 4 h, 1 M HCl was added, the reaction mixture was extracted with CH_2Cl_2, the organic layers were successively washed with saturated $NaHCO_3$ solution and brine, and after drying on Na_2SO_4, the solvent was removed under vacuum affording the sufinate esters in 95% yield. Analysis of the crude mixture in deuterated benzene revealed a diastereomeric ratio of 97:3. Pure samples of individual diastereomers were obtained by silica gel column chromatography by using 2-propanol/hexanes (1:20) as elutant, affording **23**-S_S (1.9 g, 73%) as the major diastereomer. Enough quantity of the minor diasteroisomer was obtained for analysis by repeating the reaction several times.

For the major diastereomer **23**-S_S: R_f 0.18 (hexanes/2-propanol, 20:1); $[\alpha]_D$ −55 (*c* 1.05, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 5.92 (d, 1H, J = 3.6 Hz), 4.94 (d, 1H, J = 2.75 Hz), 4.61 (d, 1H, J = 3.65 Hz), 4.59−4.53 (m, 2H, H-4 and H-5), 4.26 (dd, 1H, J = 8.5 Hz, J = 5.2 Hz), 4.20 (dd, 1H, J = 8.4 Hz, J = 6.1 Hz), 2.62 (t, 2H, J = 6.75 Hz), 2.34 (t, 2H, J = 7.3 Hz), 1.51(s, 3H), 1.46 (s, 3H), 1.44−1.36 (m, 2H), 1.41(s, 3H), 1.18(s, 3H), 1.15−1.09 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 112.4, 109.2, 104.9, 83.5, 80.3, 79.3, 72.3, 66.8, 65.5, 50.8, 28.0, 26.7, 26.6, 26.2, 25.2, 18.6; HRMS *m*/*e* calcd for $C_{16}H_{27}N_3O_7S$ $(M + H)^+$ 406.1648, found 406.1649.

For the minor diastereomer **23**-R_S: R_f 0.24 (hexanes/2-propanol, 20:1); $[\alpha]_D$ −5.6 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (d, 1H, J = 3.2 Hz, H-1), 5.04 (d, 1H, J = 3.8 Hz), 4.95 (d, 1H, J = 2.76 Hz), 4.50 (dd, 1H, J = 2.8 Hz, J = 8.5 Hz), 4.40−4.32 (m, 1H), 4.17 (dd, 1H, J = 4.5 Hz, J = 8.9 Hz), 4.10 (dd, 1H, J = 6.1 Hz, J = 8.9 Hz), 2.63 (t, 2H, J = 6.8 Hz), 2.54−2.39 (m, 2H), 1.45 (s, 3H), 1.44 (s, 3H), 1.33 (s, 3H), 1.18−1.15 (m, 2H), 1,09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 112.4, 109.4, 105.3, 83.8, 83.1, 80.8, 72.1, 67.7, 57.0, 56.5, 50.8, 28.0, 26.9, 26.7, 26.1, 25.2, 18.4.

(*R*)-(−)-1-Azido-4-(methylsulfinyl)butane (24-R_S): Sulfinate ester **23**-S_S (345 mg, 0.85 mmol) was dissolved in anhydrous toluene (14 mL). After the mixture was cooled to 0 °C, methyl magnesium chloride (0.36 mL, 5 mmol) was added and the solution was stirred for 1 h at 0 °C. The mixture was then neutralized with a saturated solution of aqueous NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 and the resulting organic layers were combined, dried on Na_2SO_4, and concentrated. Chromatography of the residue gave the sulfoxide **24**-R_S (127 mg, 93%) as a colorless liquid. R_f 0.31 (EtOAc/MeOH, 9:1); $[\alpha]_D$ −81,53 (*c* 0.16, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.33 (t, 2H, J = 6.5 Hz), 2.74−2.63 (m, 2H), 2.55 (s, 3H), 1.88−1.79 (m, 2H), 1.77−1.69 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.9, 50.9, 38.6, 28.0, 20.0; HRMS *m*/*e* calcd for $C_5H_{11}N_3OS$ $(M + H)^+$ 162.0701, found 162.0698.

(*R*)-(−)-1-(4-Azidobutylsulfinyl)Pentane (25-R_S): In a round-bottomed flask flushed with argon was placed anhydrous magnesium (17.91 mg, 0.74 mmol), which was covered with Et_2O (0.5 mL), then 1-bromopentane (0.1 mL, 0.74 mmol) was added drop by drop while maintaining the reflux of the solution affording a solution of the Grignard reagent. In a second round-bottomed flask under argon atmosphere was placed the sulfinate ester **23**-S_S (250 mg, 0.61 mmol) in toluene (10 mL) and the solution was cooled to 0 °C. Then the Grignard reagent was added to the solution of the sulfinate ester through the cannula and the mixture was stirred 1 h at 0 °C. Neutralization was then carried out with a saturated solution of aqueous NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 and the resulting organic layers were combined, dried on Na_2SO_4, and concentrated. Column chromatography of the residue gave the sulfoxide **25**-R_S (117,7 mg, 88%) as a colorless liquid. R_f 0.13 (EtOAc/CH_2Cl_2, 1: 4); $[\alpha]_D$ −7,17 (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.41−3.33 (m, 2H), 2.75−2.61 (m, 4H), 1.93−1.87 (m, 2H), 1.84−1.73 (m, 4H), 1.51−1.36 (m, 4H), 0.93 (t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.6, 51.7, 50.9, 30.9, 28.1, 22.3, 20.1, 13.8; HRMS *m*/*e* calcd for $C_9H_{20}N_3OS$ $(M + H)^+$ 218.1327, found 218.1333.

(*R*)-(−)-(4-azidobutylsulfinyl)cyclohexane (26-R_S): Sulfinate ester **23**-S_S (300 mg, 0.735 mmol) was dissolved in anhydrous toluene (12 mL). After the mixture was cooled to 0 °C, 2 M cyclohexylmagnesium chloride (0.15 mL, 0.95 mmol) was added, then the reaction mixture stirred for 1 h. Neutralization was then carried out with a saturated solution of aqueous NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 and the resulting organic layers were combined, dried on Na_2SO_4, and concentrated. Column chromatography of the residue gave the sulfoxide **26**-R_S (100 mg, 59%) as a colorless liquid. R_f 0.15 (EtOAc/DCM, 1:4); $[\alpha]_D$ −31.7 (*c* 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.42−3.33 (m, 2H), 2.72−2.63 (m, 2H), 2.60−2.54 (m, 1H), 2.14 (d, 1H, J = 13 Hz), 1.97−1.69 (m, 9H), 1.55−1.25 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 59.1, 50.9, 48.2, 28.1, 26.4, 25.5, 25.4, 25.1, 24.9, 20.3; HRMS *m*/*e* calcd for $C_{10}H_{19}N_3OS$ $(M + H)^+$ 230.1327, found 230.1326.

(*R*)-(−)-2-(4-Azidobutylsulfinyl)naphthalene (27-R_S): The Grignard reagent was freshly prepared as follows: In a round-bottomed flask flushed with argon was placed anhydrous magnesium (243.1 mg, 10 mmol), which was covered with Et_2O (8.18 mL), then 1-bromonaphthalene (1.39 mL, 10 mmol) was added drop by drop while maintaining the reflux of the mixture.

In a second flask under argon atmosphere, a solution of sulfinate ester **23**-S_S (250 mg, 0.61 mmol) in toluene (10 mL) was cooled to 0 °C. The 1-naphthylmagnesium chloride (0.6 mL) was added to a sulfinate solution through a cannula and stirred 1 h at 0 °C. Neutralization was then carried out with a saturated solution of aqueous NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 and the resulting organic layers were combined, dried on Na_2SO_4 ,and concentrated. Chromatography of the residue gave the sulfoxide **27**-R_S (69 mg, 43%) as a colorless liquid. R_f 0.34 (EtOAc/hexanes, 1:1); $[\alpha]_D$ −367.65 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (dd, 1H, J = 1 Hz, J = 7.5 Hz), 8.01−7.94 (m, 3H), 7.69 (dd, 1H, J = 7.5 Hz, J = 8.5 Hz), 7.64−7.59 (m, 2H), 3.35−3.26 (m, 2H), 3.11−2.84 (m, 2H), 2.04−1.95−171 (m, 2H), 1.82−1.77 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.2, 133.5, 131.2, 129.1, 128.8, 127.3, 126.7, 125.6, 123.1, 121.4, 54.9, 50.9, 28.0, 19.8; HRMS *m*/*e* calcd for $C_{14}H_{15}N_3OS$ $(M + H)^+$ 274.1014, found 274.1016.

(*R*)-(−)-11-(4-Azidobutylsulfinyl)Undec-1-ene (28-R_S): Sulfinate ester **23**-S_S (323 mg, 0.792 mmol) was dissolved in

anhydrous toluene (13 mL). After the solution was cooled to 0 °C, 1-undecene magnesium bromide (0.9 mL, 1.03 mmol) prepared from 11-bromo-1-undecene and magnesium powder in ether at reflux was added, and the stirring was continued for 1 h. Neutralization was then carried out with a saturated solution of aqueous NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 and the resulting organic layers were combined, dried on Na_2SO_4, and concentrated. Column chromatography of the residue gave the sulfoxide **28**-R_S (188.2 mg, 79%) as a white solid. R_f 0.15 (EtOAc/hHexanes, 1:1); $[\alpha]_D$ −5.47 (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87−5.79 (m, 1H), 5.03−4.99 (m, 1H), 4.97−4.94 (m, 1H), 3.42−3.33 (m), 2.75−2.64 (m, 4H), 2.06 (q, 2H, J = 14.5 Hz), 1.94−1.88 (m, 4H), 1.50−1.44 (m, 2H), 1.42−1.27 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.0, 114.1, 52.6, 51.7, 50.9, 33.7, 29.4, 29.3, 29.2, 29.2, 29.1, 29.0, 28.9, 28.1, 22.6, 20.1; HRMS *m*/*e* calcd for $C_{15}H_{29}N_3OS$ $(M + H)^+$ 299.1616, found 299.1621.

(*R*)-(−)-1-Isocyanato-4-(methylsulfinyl)butane (2-R_S): To a solution of *R*)-(−)-1-azido-4-(methylsulfinyl)butane **24**-R_S (105 mg, 0.67 mmol) was added triphenylphosphine (342.5 mg, 1.26 mmol) in 4.8 mL of ether. After the reaction had been refluxed for 3 h, the solvent was removed by vacuum. To this residue was added carbon disulfide (0.97 mL); after this mixture was refluxed for 1 h, the solvent was removed under vacuum. The crude product was purified by silica gel chromatography, using 9:1 EtOAc/MeOH, affording **2**-R_S (82.2 mg, 71%) as a colorless liquid. R_f 0.15 (EtOAc/MeOH, 9:1); $[\alpha]_D$ −78.2 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.62 (t, 2H, J = 10.5 Hz), 2.84−2.67 (m, 2H), 2.61 (s, 3H), 2.02−1.86 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.5, 44.6, 38.7, 29.0, 20.1; HRMS *m*/*e* calcd for $C_6H_{11}NOS_2$ $(M + H)^+$ 178.0360, found 178.0367.

(*R*)-1-(4-Isothiocyanatobutylsulfinyl)pentane (11-R_S): To a solution of 80 mg (0.37 mmol) of sulfoxide **25**-R_S was added a solution of triphenylphosphine (194.3 mg, 0.71 mmol) in ether (2.6 mL). After the reaction had been refluxed for 3 h, the solvent was removed by vacuum. To this residue was then added carbon disulfide (0.55 mL); after this the mixture was refluxed for 1 h, the solvent was removed under vacuum. The crude product was purified by silica gel chromatography, using 20:1 EtOAc/MeOH, affording 40 mg (47%) of a colorless liquid. R_f 0.31 (EtOAc/MeOH, 20:1); $[\alpha]_D$ −6.4 (*c* 1, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.61 (t, 2H, J = 6 Hz), 2.76−2.62 (m,4H), 1.98−1.82 (m, 4H), 1.80−1.73 (m, 2H), 1.51−1.34 (m, 4H), 0.93 (t, 3H, J = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.6, 51.2, 44.6, 30.9, 29.1, 22.2, 20.1, 13.8; HRMS *m*/*e* calcd for $C_{10}H_{19}NOS_2$ $(M + H)^+$ 234.0986, found 234.0988.

(*R*)-(4-Isothiocyanatobutylsulfinyl)cyclohexane (12-R_S): To a solution of sulfoxide **26**-R_S (80 mg, 0.35 mmol) was added a solution of triphenylphosphine (177.6 mg, 0.677 mmol) in ether (2.5 mL). After the reaction had been refluxed for 3 h, the solvent was removed in vacuo. To this residue was added 0.5 mL of carbon disulfide; after this the mixture was refluxed for 1 h and the solvent was removed under vacuum. The crude product was purified by silica gel chromatography, using 4:1 EtOAc/CH_2Cl_2, affording **12**-R_S (60.4 mg, 70.5%) as a colorless liquid. R_f 0.22 (EtOAc/CH_2Cl_2, 4:1); $[\alpha]_D$ −27.48 (*c* 1.14, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.61 (t, 2H, J = 5.5 Hz), 2.73−2.65 (m, 2H), 2.61−2.55 (m, 1H), 2.14 (d, 1H, J = 14 Hz), 2.00−1.72 (m, 8H), 1.54−1.24 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 59.2, 47.8, 44.7, 29.1, 26.4, 25.5, 25.4, 25.1, 24.9, 20.3; HRMS *m*/*e* calcd for $C_{11}H_{19}NOS_2$ $(M + H)^+$ 245.0908 found 245.0910.

(*R*)-2-(4-Isothiocyanatobutylsulfinyl)naphthalene (13-R_S): To a solution of sulfoxide **27**-R_S (60 mg, 0.22 mmol) was added a solution of triphenylphosphine (115.4 mg, 0.42 mmol) in ether (1.55 mL). After the reaction had been refluxed for 3 h, the solvent was removed in vacuo. To this residue was added carbon disulfide (0.33 mL); after this mixture was refluxed for 1 h, the solvent was removed under vacuum. The crude product was purified by silica gel chromatography, using 1:2 EtOAc/hexanes, affording **13**-R_S (57.2 mg, 90%) as a colorless liquid. R_f 0.31 (EtOAc/hexanes, 1:2); $[\alpha]_D$ −377.5 (*c* 1, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (dd, 1H, J = 1 Hz, J = 7 Hz), 8.00−7.92 (m, 3H), 7.68 (dd, 1H, J = 7.5 Hz, J = 8.5 Hz), 7.62−7.57 (m, 2H), 3.54−3.51 (m, 2H), 3.10−2.81 (m, 2H), 2.02−1.74 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.0, 133.5, 131.2, 129.2, 128.7, 127.4, 126.7, 125.6, 123.1, 121.3, 54.3, 44.6, 29.0, 19.7; HRMS *m*/*e* calcd for $C_{15}H_{15}NOS_2$ $(M + H)^{2+}$ 290.0673, found 290.0656.

(*R*)-11-(4-Isothiocyanatobutylsulfinyl)undec-1-ene (14-R_S): To a solution of sulfoxide **28**-R_S (131 mg, 0.437 mmol) in ether (3 mL) was added triphenylphosphine (222.3 mg, 0.847 mmol). After the reaction had been refluxed for 3 h, the solvent was removed under vacuum. To this residue was added carbon disulfide (0.65 mL); after this mixture was refluxed for 1 h, the solvent was removed under vacuum. The crude product was purified by silica gel chromatography, using 20:1 EtOAc/hexanes, affording **14**-R_S (75.6 mg, 55%) as a white solid. R_f 0.22 (EtOAc/hexanes, 20:1); $[\alpha]_D$ −3.4 (*c* 1.0, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87−5.79 (m, 1H), 5.03−4.99 (m, 1H), 4.97−4.94 (m, 1H), 3.63 (t, J = 14.5 Hz), 2.77−2.65 (m,4H), 2.06 (dd, J = 14.5 Hz, J = 6.5 Hz), 1.99−1.87 (m, 4H), 1.85−1.78 (m, 2H), 1.53−1.27 (m, 14H); ^{13}C NMR (125 MHz,$CDCl_3$) δ 139.1, 114.1, 52.6, 51.7, 44.6, 33.7, 29.3, 29.2, 29.1, 29.0, 28.9, 28.8, 22.6, 20.1; HRMS *m*/*e* calcd for $C_{16}H_{29}NOS_2$ $(M + H)^{2+}$ 315.1691, found 315.1670.

Biological Activity of Sulforaphane and Sulforaphane Analogues. i. Construction of a rat NQO1-ARE luciferase reporter plasmid: The rat NQO1-ARE luciferase reporter plasmid (pGL3-rNQO1 ARE) was generated by using the pGL3-Promoter vector (Promega), which includes an SV40 promoter upstream of the luciferase gene. A double stranded oligonucleotide was synthesized containing a part of the rat *Nqo1* promoter (5̃-CTCTAGAGTCACAGTGACTTGGCAAAATCTGAC-3̃), including the antioxidant response element (underlined).[34] The sequence was extended (bold) to generate 5′-terminal *Sac*I and 3′-terminal *Xho*I restriction sites. After annealing of the complementary oligonucleotides the double stranded oligonucleotide was inserted into the vector by using the *Sac*I and *Xho*I restriction sites in the multiple cloning site upstream of the SV40 promoter.

ii. Transient transfection and luciferase reporter assays: HaCaT cells were cultured in Dulbeccòs Modified Eagle Medium (DMEM) containing 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. Transfection was performed with linear polyethylenimine (Polysciences). HaCaT cells were plated at a density of 2×10^5 cells/well in 12-well plates (Nunc) in 1 mL of DMEM, 10% FCS, 1% penicillin/streptomycin and grown to 80% confluency (24 h). Cells were washed twice with PBS, placed in 1 mL of Opti-MEM I (GIBCO), and cotransfected with 2 μg of the rNQO1-ARE luciferase reporter plasmid and 0.01 μg of the phRL-CMV *Renilla* luciferase vector plasmid (Promega) as an internal control for transfection efficiency. Both plasmid DNAs were diluted with 106 μL of 0.9% (w/v) sodium chloride solution, then 3.8 μL of polyethylenimine solution (1 mg/mL) was then added and the mixture was briefly vortexed. The mixture was then incubated at room temperature for 15 min to allow complex formation. Afterward the transfection mix was added to the cells. After 4 h the transfection mix was fully replaced by fresh cell culture medium (DMEM, 10% FCS, 1% penicillin/streptomycin). After 24 h the cells were washed with PBS and incubated with fresh culture medium containing different concentrations of the sulforaphane analogues (50 nm, 0.5 μM, 5 μM, 50 μM, 500 μM) or DMSO as negative control. Cells were harvested 20 h later, using Passive Lysis Buffer (Promega). The firefly and *renilla* luciferase activities were measured in the supernatants by using the Dual-Luciferase Reporter Assay System

kit from Promega according to the manufacturer's instructions. Luciferase activity was determined in a MicroLumatPlus LB96 V (EG&G Berthold) and reported as *x*-fold activation compared to DMSO-treated cells. Two experiments were performed in triplicate, and the results show mean ± SD from one experiment.

Acknowledgment. Financial support from the DGICyT (grant nos. CTQ2006-15515-CO2-01 and CTQ2007-61185) and the Junta de Andalucía (grants P06-FQM-01852 and P07-FQM-2774) is gratefully acknowledged. A.A. thanks the Ministerio de Ciencia e Innovacion for a Ramón y Cajal contract. F.L. is supported by a predoctoral fellowship of the "Studienstiftung des Deutschen Volkes".

Supporting Information Available: Copies of ^{1}H NMR and ^{13}C NMR spectra of compounds **2**-R_S, **11**-R_S, **12**-R_S, **13**-R_S, **14**-R_S, **15**-R_S, **16**-R_S, **17**-R_S, **18**-R_S, **23**-S_S, **23**-R_S, **24**-R_S, **25**-R_S, **26**-R_S, **27**-R_S, and **28**-R_S. This material is available free of charge via the Internet at http://pubs.acs.org.