Sulforaphane Homologues: Enantiodivergent Synthesis of both Enantiomers, Activation of the Nrf2 Transcription Factor and Cytotoxic Activity.

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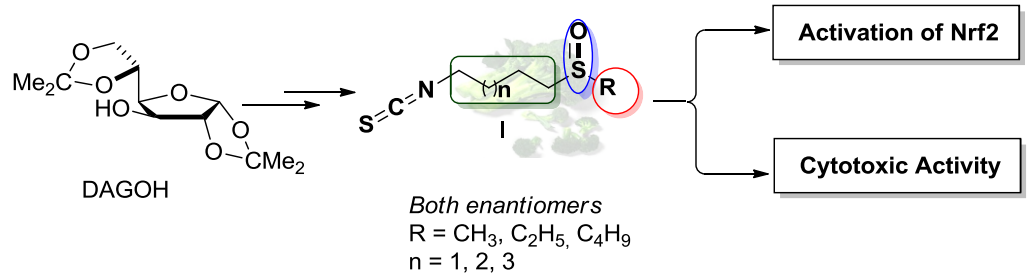
Graphical Abstract



Highlights

- Both enantiomers of sulforaphane and six homologues were obtained.
- The ability of the synthesized compounds to activate the Nrf2 is studied
- The cytotoxic activity of the analogues against cancer and healthy cells is reported.

Sulforaphane Homologues: Enantiodivergent Synthesis of both Enantiomers, Activation of the Nrf2 Transcription Factor and Selective Cytotoxic Activity.

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Abstract: Reported is an enantiodivergent approach for the synthesis of both enantiomers of sulforaphane (SFN) homologues with different chain length between the sulfinyl sulfur and the isothiocyanate groups and different substituents on the sulfinyl sulfur. The homologues were designed in order to unravel the effect of all the diversity elements included in sulforaphane's structure. The key step of the approach is the diatereoselective synthesis of both sulfinate ester epimers at sulfur, using as single chiral auxiliary the sugar derived diacetone-D-glucose. The approach allows the first synthesis of both enantiomers of 5-methylsulfinylpentyl isothiocyanate, and the biologically important 6-methylsulfinylhexyl isothiocyanate (6-HITC) found in Japanese horseradish, wasabi (Wasabia japonica). The ability of the synthesized compounds as inductors of phase II detoxifying enzymes has been studied by determining their ability to activate the cytoprotective transcription factor Nrf2. The cytotoxic activity of all the synthesized compounds against human lung adenocarcinoma (A549) and embryonic lung fibroblasts (MRC-5) is also reported.

Keywords: (*R*)- and (*S*)-Sulforaphane and Homologues; Enantiodivergent synthesis, Activation of Nrf2 factor; cytotoxic activity, DAG-methodology.

1. Introduction.

In the last years, there are more and more evidences that support the intimate relationship between nutrition and health [1]. This relationship was already known from classical Greece, when Hippokrates, 500 BC, recommended "Let your food be your medicine, and your medicine be your food". Centuries later Cervantes in Don Quixote argued that "the health of the whole body is forged in the office of the stomach". Re-awareness in modern medical science on the importance of certain foods to maintain health, prevent and alleviate diseases has induced an explosion of research on dietary phytochemicals and brought up new concepts and terminologies [2,3]. Among the star foods, cruciferous vegetables, rather than vegetables as a group, have drawn a great deal of attention because of their excellent activities in cancer prevention [4].

In this sense, epidemiological and clinical studies have established that populations with a diet rich of broccoli and other cruciferous vegetables, such as cauliflower, watercress, Brussels sprouts, and cabbage are less prone to develop certain types of cancers as well as some chronic degenerative diseases [5]. Compelling evidences indicate that the chemical carcinogenesis prevention and chemotherapeutic benefit of cruciferous are attributable to their high contents of phytochemicals containing an isothiocyanate functional group [6]. These organic isothiocyanates are not produced as such by the plant, but they result from the enzymatic action of the thioglycosidase myrosinase on natural glucosinolates (β-thioglucoside-N-hydroxysulfates), such as glucopharin **1**, Scheme 1 [7].

Scheme 1. Biosynthetic pathway to (R_S)-sulforaphane **2** and (R_S, S_C)-sulforaphane-cysteine analogue **3**: Lossen rearrangement through myrosinase deglycosylation of glycopharin **1** followed by mercapturic acid pathway.

Myrosinase is released from intracellular vesicles following mechanical damage to the plant tissue, such as that caused by chewing, food preparation, or damage by insects.[7] Myrosinase catalyses the hydrolysis of the thioglycosidic bond leading to unstable intermediates, which at physiological pH, predominantly undergo Lossen rearrangements to the corresponding isothiocyanate, such as **2** [9]. (R_S)-1-isothiocyanato-4-(methylsulfinyl)-butane (sulforaphane **2**), firstly isolated in 1992 from broccoli extracts [10], is the main inducer of phase II detoxifying enzymes and is well documented as a powerful chemopreventive agent [11]. In this regard, animal studies on rats have established the chemopreventive activity of sulforaphane against colon cancer [12], and recent preclinical and clinical studies have confirmed the chemopreventive activity of sulforaphane in women at risk for breast cancer [13]. In addition to increasing cellular capacity for detoxifying electrophiles and oxidants, SFN has been shown to induce apoptosis, and to inhibit cell cycle progression and angiogenesis [14-19]. Most recently, SFN has also been shown to induce anti-proliferative effects via epigenetic mechanisms [20], namely acting as dietary histone deacetylase inhibitor through its metabolite SFN-cysteine **3** [21, 22], and to suppress DNA methylation [23].

In nature, besides the common glycone moiety, the glycosinolate molecules possess a variable side chain derived from amino acids [24], which in the case of sulforaphane is characterized by the presence of a methylsulfinyl group. The presence of the sulfinyl group is not limited to SFN, but has been described for other isothiocyanates such as iberin (1-isothiocyanato-3-(methylsulfinyl)-propane) [25], 1-isothiocyanato-6-(methylsulfinyl)-hexane (6-HITC) [26], 1-isothiocyanato-7-(methylsulfinyl)-heptane (7-HITC), and 1-isothiocyanato-8-(methylsulfinyl)-octane (8-OITC) [27]. Among these homologues 6-HITC, found in Japanese horseradish, wasabi (Wasabia japonica, *syn.* Eutrema wasabi), is rapidly absorbed by the body and induces hepatic phase II detoxification enzymes more potently than SFN. Based on these results, in the last years there has been an intense research for the determination of the relation structure-activity of SFN, with the aim of finding biologically more active and chemically more stable analogues [28-32]. These researches were dedicated primarily to the simplification of SFN structure by using a rigid core between the sulfinyl sulfur and the isothiocyanate group for instance, or by eliminating or replacing the chiral sulfinyl group. Recently, researches were directed toward the modulation of the isothiocyanate reactivity, by using a less electrophilic functionality [33]. The data accumulated so far, indicate that the structural characteristics of natural isothiocyanate play a key role in their biological activities, as very small changes induce an important effect on the chemopreventive activities. In this regard, it has been shown that the oxidation state of the sulfur is important as both, the thioether and the sulfone analogues are less active than the corresponding sulfoxide derivatives. With regard to the sulfur chirality, it should be indicated that natural sulforaphane, iberin, and most probably all methyl sulfinyl analogues exist as a single enantiomer with an R_S absolute configuration [34], as the oxidation of the sulfide in the gluosinolates by flavin monooxygenase has been recently

reported to be stereospecific [35]. Nevertheless, up to now few studies have addressed the sulfur chirality importance on the biological activity of SFN [36]. In this sense, while original data indicate that the sulfur chirality has no significant biological effect, a recent study shows that natural *R*-sulforaphane is a far more potent inducer of the carcinogen-detoxifying enzyme systems in rat liver and lung than the unnatural *S*-isomer [37]. A subsequent study on the modulation of glucuronosyl transferase and epoxide hydrolase, two major carcinogen-metabolising enzyme systems, showed that the *R*-enantiomer enhanced, whereas the S-enantiomer impaired, glucuronosyl transferase activity and that the *R*-sulforaphane was more effective than the *S*-enantiomer in up-regulating microsomal epoxide hydrolase [38]. Finally, while the nature of the chain linking the electrophilic isothiocyanate group and the Lewis basic sulfinyl moiety on their anticancer activity has been determined, only few studies have been conducted on the importance of the substituent at the sulfinyl sulfur [31, 32, 39]. It should be noted that despite the great efforts made, and the hundreds of analogues assayed, no one surpassed yet the biological activity of natural SFN.

Figure 1: Structure of sulforaphane and homologues synthesized in this work.

Based on these premises, and as a part of our research program on sulforaphane analogues, we decided to study the effect of all the diversity elements enclosed in sulforaphane generic structure **I** (Figure 1), namely: (*i*) the chain length between the sulfinyl sulfur and the isothiocyanate group, (*ii*) the steric hindrance of the substituent at sulfur, and (*iii*) the sulfur stereochemistry. Therefore, herein we report a convergent and high yielding approximation to both isomers of sulforaphane (**2***R*$_S$, **2***S*$_S$) and analogues **4***R*$_S$, **4***S*$_S$, **5***R*$_S$, **5***S*$_S$, **6***R*$_S$, and **7***R*$_S$, Figure 1, with different

substituents at the sulfinyl sulfur, varied alkyl chain length between the two functional groups, and in both enantiomeric forms. The biological activity of these compounds as inductors of phase II detoxifying enzymes has been studied by determining their ability to activate the cytoprotective transcription factor Nrf2. The anti-cancer activity has been determined by evaluating their selective cytotoxic effect in human lung adenocarcinoma (A549) and non-malignant lung cells (MRC-5).

2. Results and discussion

2.1. Chemical synthesis

The last decade has witnessed a renewed interest towards 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 bond formation [40, 41]. Additionally, besides sulforaphane, there are a number of pharmacologically relevant drugs [42], which have in their structure a sulfinyl group including the proton pump inhibitor esomeprazole [43], the vigilance promoting armodafinyl [44],[37] and the anti-inflammatory sulindac [45].

Although the natural sulforaphane exists as a single enantiomer in nature, most studies conducted on its biological activities have been conducted using the racemic form. As an alkyl methyl sulfoxide, the synthesis of enantiopure sulforaphane is not straightforward [46-49], and the main approximations for the synthesis of enantiomerically pure sulfoxides developed so far fail in the preparation of simple dialkyl sulfoxides. Actually, there is no asymmetric catalytic oxidation able to give

enantiomerically pure dialkyl sulfoxides from the corresponding prochiral thioethers [50]. In the particular case of sulforaphane, only four approximations have been reported so far, two of them give the natural enatiomer in scalemic form. The first one, developed by Holland's group, used a microbial oxidation with Helmithosporium *sp* of the corresponding prochiral sulfide, which afforded R_S-sulforaphane in 86% ee and 54% yield [51]. Whitesell's group used *trans*-2-phenyl hexanol as chiral auxiliary for the synthesis of diasteromerically pure methyl sulfinate ester **8**, from which R_S-sulforaphane was obtained through 5 steps and 42% overall yield [52]. Schenk's group employed a method based on a diastereoselective oxidation of cationic ruthenium complex **9**, which leads to R_S-sulforaphane with a good 80% ee [53].

Figure 2: Diastereomeric intermediates used for the synthesis of enantiopure and scalemic R_S-sulforaphane

Noteworthy, none of these methods is able to give sulforaphane analogues with substituent at the sulfur different from a methyl group. In order to solve this problem we have recently reported that sulfinate ester **10**, is an effective sulfinylating agent able to efficiently transfer the linear alkyl sulforaphane side chain in enantiomeric form [39]. In the present work, in order to modulate the different variables of sulforaphane, we need a methodology capable of producing sulforaphane homologues with different substituents at the sulfur, and different chain length between the sulfoxide and the isothiocyanate groups, preferably in both enantiomeric

forms. In this regard, the "DAG-methodology" [54], developed in our group, seems the method of choice as it is able to give a diasteromerically pure sulfinate ester with a non-hindered alkyl chain at the sulfinyl sulfur. Additionally, based on our experimental and theoretical studies [55], both epimers at sulfur should be accessible in an enantiodivergent manner, by a simple change of the base used to catalyse the reaction, from the corresponding sulfinyl chlorides [56].

For the synthesis of the desired 1-azidobutanesulfinyl chloride, 1-azidopentanesulfinyl chloride, and 1-azidohexanesulfinyl chloride, two approaches were used, starting either from the corresponding chloro-alcohols **11**, **13** or from the diol **12**, Scheme 2.

Scheme 2: Synthesis of 1-azido-butanesulfinyl chlorides **23-25**. a) NaN_3, DMF, b) MsCl, NEt_3, CH_2Cl_2, c) KSAc, DMF, d) SO_2Cl_2, Ac_2O, CH_2Cl_2.

Treatment of 1-chlorobutanol **11** or 1-chlorohexanol **13** with sodium azide in DMF afforded the azido alcohols **14** and **16** in 73% and 98% yields respectively, which after mesylation gave the azido mesylate intermediates **17** and **19** in excellent yields. Alternatively, a double mesylation of 1,5-pentanediol **12**, afforded the dimesylated compound **15**, which was de-symmetrized to give compound **18** by reaction with sodium azide in DMF. Treatment of the mesylates **17, 18** and **19** with sodium thioacetate afforded compounds **20, 21** and **22** in 84%, 82% and 85% yields, respectively. The direct transformation of thioacetates **20-22** to the desired sulfinyl chlorides **23, 24** and **25** has been achieved by treatment with sulfuryl chloride and acetic anhydride in methylene chloride [57].

Once the sulfinyl chlorides in hand, we conducted the diastereodivergent syntheses of the corresponding DAG sulfinate esters using our "DAG methodology". In this methodology, a single inducer of chirality, Diacetone-D-glucose (DAG), is used for the stereoselective synthesis of both diastereomerically pure (*R*)- and (*S*)-sulfinate esters, by dynamic kinetic resolution of the corresponding sulfinyl chloride, thanks to the opposite stereodirecting effect of the base used to catalyze the reaction [51]. In this sense, using Hunig-base, (S_S)-DAG sulfinate esters are generally obtained in good to high chemical yields and *d.e's*, while the use of pyridine as base afforded the epimers at sufur (R_S)-DAG sulfinate esters. Theoretical studies at the ONIOM (Beckel3LYP:UFF) level, reproduced the experimental results and showed that the base used plays a dual role, catalyzing the interconversion of both enantiomers of sulfinyl chlorides, and assisting the displacement of the chlorine by DAGOH. The analysis of the optimized geometries revealed that the most sterically relevant substituent around the sulfur is the R group of the substrate with pyridine as base, but the base itself with DIPEA. This leads to an inversion of the chiral distribution of steric hindrance around sulfur that induces the reversal of the stereochemical outcome and the enantiodivergency observed [54].

Scheme 3: Enantiodivergent synthesis of (*S*)- and (*R*)-sulfinate esters **10**, **26**, and **27** using the DAG-methodology

In accord with the theoretical studies, the treatment of DAG with azidoalcanesulfinyl chlorides **23, 24** and **25**, in the presence of DIPEA afforded the

(*S*)- sulfinate esters **10**-S_S**, 26**-S_S **,** and **27**-S_S, with 92%, 92% and 87% yields and a good 94%, 88% and 84% de's; although lower than that obtained with other sulfinyl chlorides, which are usually obtained as a single diastereoisomer. Interestingly, when pyridine was used as base, (*R*)-sulfinate esters **10**-R_S**, 26**-R_S**,** and **27**-R_S, were predominantly obtained in 90%, 87%, and 86% yields respectively, although in a lower diastereomeric excesses, Scheme 3. The diastereoselectivity of all the reactions has been easily determined by ^{1}HNMR analysis of the crude, employing deuterated benzene as solvent. In all sulfinates esters, the use of C_6D_6 induces a splitting of the anomeric protons, as well as that of the protons in the two and three positions of the S_S-sulfinate esters and their epimers the R_S-sulfinates (see supporting information). Additionally, all the diastereomeric sulfinate esters possess a good separation factor, which allows their preparation in optically pure form by column chromatography.

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 esters are the intermediates, which will lead to sulforaphane and sulforaphane homologues with the same absolute configuration as the natural sulforaphane, while R_S-DAG sulfinate esters will afford unnatural homologues.

Scheme 4: Synthesis of enantiopure natural sulforaphane **2**-R_S and homologues **4**-R_S and **5**-R_S: a) CH_3MgBr, toluene b) (i) PPh_3, Et_2O, reflux, (ii) CS_2, reflux

In this sense, condensation of methyl Grignard on the sulfinate ester **10**-S_S, **26**-S_S and **27**-S_S afforded the corresponding azidoalkyl methyl sulfoxides **28**-R_S, **29**-R_S, and

30-R_S in 90%, 74% and 73% yields, respectively, Scheme 4. Staudinger reaction of the azido derivatives with triphenylphosphine and subsequent aza Wittig-type condensation of the resulting iminophosphorane with carbon disulfide lead to enantiomerically pure natural (-)-sulforaphane **2**-R_S, and for the first synthesis of enantiopure homologues **4**-R_S, and **5**-R_S in 98%, 91% and 81% yields, respectively, Scheme 4. Similarly, condensation of methyl Grignard on the sulfinate ester **10**-R_S, **26**-R_S and **27**-R_S afforded the corresponding 4-azidobutyl methyl sulfoxides **28**-S_S, **29**-S_S, and **30**-S_S in 87%, 78% and 81% yields, respectively, Scheme 5. After Staudinger reaction and subsequent aza Wittig-type condensation of the resulting iminophopsphorane with carbon disulfide lead to enantiomerically pure unnatural (+)-sulforaphane **2**-S_S, and for the first synthesis of the enantiopure homologues **4**-S_S, and **5**-S_S in 78%, 97% and 87% yields respectively, Scheme 5.

Scheme 5: Synthesis of enantiopure unnatural sulforaphane **2**-S_S and homologues **4**-S_S and **5**-S_S: a) CH_3MgBr, toluene b) (i) PPh_3, Et_2O, reflux, (ii) CS_2, reflux

We have previously reported data, pointing out that the steric hindrance of the substituent at the sulfinyl sulfur may strongly affect the activation of the cytoprotective Nrf2 transcription factor. In order to complete these studies and to know to what extent we can increase the size of the sulfur substituent without affecting the biological activity of sulforaphane, we designed compounds **6**-R_S and **7**-R_S with the same absolute configuration at sulfur than the natural (-)-sulforaphane, but having an ethyl and butyl substituent instead of a methyl group.

Condensation of the adequate Grignard reagent on sulfinate ester **10**-S_S lead to the desired azido sulfoxides **31** and **32**. A subsequent 2-reactions-one pot sequence leads as before to the enantiopure sulfinyl isothiocyantes **6**-R_S and **7**-R_S in 95% and 98% yields respectively, Scheme 6.

Scheme 6: Synthesis of enantiopure sulforaphane analogues **6**-R_S and **7**-R_S: a) C_2H_5MgBr, toluene b) C_4H_7MgBr, toluene, c) (i) PPh_3, Et_2O, reflux, (ii) CS_2, reflux

2.2. Biological activities of sulforaphane homologues

2.2.1 Activation of Nrf2

Experimental evidences indicate that sulforaphane exerts its anti-cancerogenic activity through the activation of "nuclear factor erythroid derived 2, like 2" (Nrf2) [58]. Nrf2 is a transcription factor, which controls the expression of a battery of cytoprotective genes in humans and animals [59]. However, unlike many other transcription factors, the major path of activation is not controlled by a kinase, but by direct covalent modification of the Nrf2 inhibitor Keap1 by electrophilic compounds. Activated Nrfd2 dimerizes with small Maf proteins or other leucine zipper proteins and binds as a heterodimer to the antioxidant response element (ARE) in the promoter or enhancer 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

dehydrogenase quinone 1 (NQO1), several glutathione S-transferases (GST), γ-glutamylcysteine synthetase (γ-GCS), peroxiredoxin 1, and heme oxygenase 1 (HO-1) [60]. The prevailing hypothesis for sulforaphanes's cellular mechanism is that the natural product covalently modifies Keap1 on one or more of its 27 cysteine residues, altering the Keap1-Nrf2-Cullin-3 protein complex [61]. This allows stabilization and accumulation of newly synthetized Nrf2 in the nucleus, where it can activate the expression of its target genes.

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, we have recently emitted the hypothesis that the function of the sulfinyl group, which has not been determined yet, must be that of optimizing the side interaction of sulforaphane 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 groups are less active. In accord with this hypothesis we have recently shown that the steric hindrance of the substituent at the sulfinyl sulfur have a substantial effect on the biological activity.

Figure 3: Dose dependent effects of sulforaphane analogues on the activation of an ARE reporter gene. HaCaT keratinocytes were transiently transfected with a rat NQO1-ARE luciferase reporter plasmid and the phRL-CMV *Renilla* luciferase vector. Transfected cells were incubated for 24h with different concentrations of sulforaphane **2**-R_S and homologues **4**-R_S**,** **4**-S_S **5**-R_S**,** **5**-S_S, **6**-R_S**,** and **7**-R_S**.** Results

were normalized to the *renilla* luciferase activity. Values show mean +/- S.D. from triplicate determinations. The result was reproduced in an independent transfection experiment.

The ability of natural sulforaphane and homologues **4**-R_S**,** **4**-S_S**,** **5**-R_S**,** **5**-S_S**,** as well as **6**-R_S**,** and **7**-R_S to activate Nrf2 was investigated 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) [62, 63]. The transfected cells were treated with five different concentrations of sulforaphane analogues (50nM, 0.5µM, 5µM, 50µM, and 500µM), and the results are given in figure 3. The concentrations 50µM and 500µM 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 homologues, being equal or more active compared to the original compound. The activation of Nrf2 was intimately related to the length of the alkyl chain between the isothiocyanate and the sulfoxide group. With regard to the substituent at the sulfinyl sulfur dialkyl sulfoxides **6**-R_S and **7**-R_S, have the same activity as natural sulforaphane. Taking into account that in our previous study we have shown that analogue with a pentyl group at sulfur was less active than SFN; we can conclude that the largest group, which can replace the methyl group of sulforaphane, is a butyl group. On the one hand, if we consider the statistical studies, although there is no noticeable difference between the ARE-activating activity of different homologues and sulforaphane **2**-R_S, we can see that the increase in the length of the carbon chain separating the isothiocyanate

group sulfinyl sulfur from 4 to 5 to 6 carbon atoms positively influences the ARE-activating activitiy of the homologues. Also, comparing the results obtained with compounds **4**-R_S and **5**-R_S, with those obtained with their enantiomers **4**-S_S and **5**-S_S, we can conclude that the configuration at sulfur plays no role in the activation of Nrf2. Therefore, the most important conclusion from the study of this family of homologues is that both enantiomers of 1-isothiocyanato-6-(methylsulfinyl)-hexane **5** (6-HITC), found in Japanese horseradish, wasabi (Wasabia japonica, syn. Eutrema wasabi), are at least equally or even more potent than the natural sulforaphane.

2.2.2. SELECTIVE CYTOTOXICITY FOR CANCER CELLS

As already mentioned, natural (R_S)-sulforaphane is not only able to prevent, but may also help to cure several types of cancer [14-18]. In order to test the cytotoxic activity for cancer cells as well as the selectivity and safety toward normal cells of our synthesized analogues, we have performed cytotoxicity assays. For this study, we used the human adenocarcinoma A549 cell line as cancer cells and fetal lung fibroblasts (MRC-5 cell line) as normal cells. The cell viability has been quantified by the colorimetric MTT assay, which measures the mitochondrial dehydrogenase activity [64, 65]. The cytotoxicity and selectivity of the synthetic analogues were quantified by determining the IC_{50} value of each compound for cancerous and normal cells, and the results are given in table 1. As a negative control, we evaluated the cytotoxic activity of lactic acid (Table 1, entry 1), together with cisplatin, a clinically used drug as a positive control (Table 1, entry 2).

Table 1. Selective cytotoxic effect of sulforaphane analogues on tumoral (A549) and normal (MRC-5) cells

These results indicate that natural sulforaphane show some selectivity against the tumor cell line, as its IC_{50} for the healthy lung cell line is more than double than for the tumoural cell line (Table 1, entry 3). With regard to the effect of chain length between the sulfoxide and the isothiocyanate group, although no significant differences in cytotoxic activity of the analogues was seen when we pass from five (Table 1, entries 4-5) to six carbon atoms (Table 1, entries 6-7), it is important to note that in both cases, a decrease of the IC_{50} compared to sulforaphane was observed. These results indicate that the synthesized analogues are more effective against lung cancer cells than natural sulforaphane. It is precisely at concentrations near these IC_{50} (10 μ M) where toxicity difference of these products occurs, because a 50% of cell inhibition was observed for the cancer cell line, only a 20% inhibition was seen for the normal cells, Figure 4. The same trend was observed in the case of the derivative **7**-R_S, analogue (Table 1, entry 7) where the methylsulfinyl group was substituting an *n*-butylsulfinyl. Not only a decrease in IC_{50} (A549) was observed up to values close to 10 μM, but at the same concentration, about 90% cell viability was maintained for the healthy lung cell line. Furthermore, the analogue **6**-R_S with an ethylsulfinyl group have an anticancer activity similar to that of sulforaphane (Table 1, entry 8), but turns out to be somewhat less toxic to normal lung cells. Finally, from all tested analogues only **5**-S_S (Table 1, entry 7) could match the slight

selectivity of SFN (Table 1, entry 7) towards the cancer cells, which was 2.3-fold and lower than that of the anticancer agent cisplatin.

Figure 4. Inverted microscope images corresponding MRC-5 and A549 cell lines following treatment with **5**-S_S analogue at a 10 μM concentration

3. CONCLUSIONS

In conclusion, we have developed a convergent and highly efficient approximation for the synthesis of both enantiomers of sulforaphane and six of its analogues with different chain length between the sulfinyl sulfur and the isothiocyante group, and different substituents at the sulfinyl sulfur. The efficiency of the obtained homologues as inductors of phase II detoxifying enzymes was determined by studying their ability to activate the cytoprotective transcription factor Nrf2. The results obtained indicate from one side, that lengthening the alkyl chain between the sulfinyl sulfur and the isothiocyanate group from 4 to 6 carbon atoms has a beneficial effect on the activation of Nrf2. Conversely, increasing the steric size of the substituent on the sulfur has a negative effect on the biological activity, the ethyl being the largest group allowed to replace the methyl substituent. The identical results obtained with both enantiomers of the compounds tested, indicates that sulfur stereochemistry has no effect on the ability of these analogues to activate the cytoprotective transcription factor Nrf2. Cytotoxicity activity studies show that all tested analogues are cytotoxic for cancer cells. Relation structure activity shows that

as in the case of Nrf2 activation, lengthening the alkyl chain between the sulfinyl sulfur and the isothiocyanate has a beneficial on the cytotoxic activity, and that the sulfur stereochemistry has no effect. Conversely, increasing the steric size of the substituent on the sulfur has a positive effect on the cytotoxic activity, the analogue with butyl group being the most cytotoxic compound.

4. Experimental Section

General experimental 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 (Merck) with detection by charring with phosphomolybdic acid/EtOH. For flash chromatography, silica Gel (Merck 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 a Bruker Avance DPX300 (1H, 300 MHz) and Bruker Avance DRX500 (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. High-resolution mass spectra were recorded on a Kratos MS-80RFA 241-MC apparatus. The organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuum.

4.1. Chemistry

4-azidobutan-1-ol (14).

A suspension of 4-chlorobutanol **11** (5.1 g, 47 mmol) and sodium azide (9.16 g, 141 mmol) in water (10 mL) were placed in a 10-20 mL crimp-sealed microwave vial equipped with a magnetic stirrer. The reaction tube was placed inside the cavity of a Biotage Initiator Mmicrowave Synthesizer, operated at 130 ºC, power 50 Watt and pressure 7-9 bar for 30 minutes. After completion of the reaction, the aqueous layer was extracted with diethyl ether twice. The resulting organic layers were combined, dried on Na_2SO_4, and concentrated under vacuum to give 3.95 g (73 %) of **14** as colourless oil. R_f = 0.35 (CH_2Cl_2/EtOAc, 92:1); ^{1}H NMR (500 MHz, $CDCl_3$) 3.70 (t, 2H, *J* = 6Hz), 3.35 (t, 2H, *J* = 6.5Hz), 1.72-1.67 (m, 4H), 1.54 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 62.2, 51.3, 29.7, 25.4.

5-methylsulfonyloxypentyl methanesulfonate (15).

To a solution of 1,5-pentanediol **12** (5.66 g, 54.4 mmol) and EtN_3 (16.7 mL, 119 mmol) in THF (80 mL) at 0ºC was added dropwise methanesulfonyl chloride (11.7 mL, 119 mmol). After 1h at 0ºC, 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 1:1 Hexanes/EtAcO, affording 12.73 g (90%) of **15** as colourless liquid. R_f = 0.50 (Hexanes/EtAcO, 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.23 (t, 2H, *J* = 6.3 Hz), 3.00 (s, 3H), 1.84-1.75 (m, 4H), 1.64-1.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 69.5, 37.4, 28.5, 21.6; HRMS (FAB) calcd. or $C_7H_{17}N_3O_6S_2$ $(M+Na)^+$: *m/e* 261.0467, found *m/e* 261.0465.

6-azidohexan-1-ol (16).

To a solution of 6-chlorohexan-1-ol **13** (15 g, 109.80 mmol) in dry DMF (50 mL) was added sodium azide (22.83 g, 351.36 mmol) and 18-crown(6) (0.77 mmol) . The reaction mixture was heated to 70 ºC overnight. The NaN_3 was filtered and the solvent was removed by vacuum. The residue was resuspended in water and extracted with Et_2O twice. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to afford 15.4 g (98% yield) of **16** as colourless oil, which was used without further purification. An analytical sample was purified by column chromatography (silica gel) for characterization employing 7:3 CH_2Cl_2/Hexanes as eluent. R_f = 0.35 (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) 3.63 (t, 2H, *J* = 6.5 Hz), 3.26 (t, 2H, *J* = 6.9 Hz), 1.65-1.52 (m, 4H), 1.50 (sa, 1H), 1.42-1.37 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 62.7, 51.3, 32.5, 28.7, 26.5, 25.3; HRMS (FAB) calcd. for C_6H13N_3O $(M)^+$ *m/e* 143.1059, found *m/e* 143.1059.

Synthesis of azidoalkyl methanesulfonate 17 and 19 from azidoalkylalcochols. General Procedure.

To a solution of the azidoalkylalcohol and triethylamine (110 mol%) in methylene chloride, at 0 ºC, was added methanesulfonyl chloride (110 mol%) dropwise. After 1.5 h at 0ºC, the reaction mixture was quenched with saturated NH_4Cl aqueous solution and extracted with CH_2Cl_2 twice. Then the combined organic layers were dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography to give the desired product.

4-azidobutyl methanesulfonate (17). It was prepared following the general procedure from 4-azidobutan-1-ol **14** (3.95 g, 34.3 mmol). Purified by Flash Chromatography (2:1, CH_2Cl_2/Hexanes) gave 4.76 g (72% yield) of **17** as colourless oil. R_f = 0.54 (EtOAc/ Hexanes, 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.27 (t, 2H, *J* =

6.3Hz), 3.36 (t, 2H, *J* = 6.6Hz), 3.02 (s, 3H), 1.91-1.72 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 69.12, 50.74, 37.44, 26.47, 25.05.

6-azidohexyl methanesulfonate (19). It was prepared following the general procedure from 6-azido-hexan-1-ol **16** (15.4 g, 107.7 mmol). Purified by Flash Chromatography (4:1, CH_2Cl_2/Hexanes) gave 23.4 g (98% yield) of **19** as colourless oil. R_f = 0.25 (CH_2Cl_2/Hexanes, 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.22 (t, 2H, *J* = 6.4 Hz), 3.27 (t, 2H, *J* = 6.8 Hz), 3.00 (s, 3H), 1.81-1.72 (m, 2H), 1.66-1.55 (m, 2H), 1.50-1.37 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 69.7, 51.2, 37.3, 28.9, 28.6, 26.1, 25.0; HRMS (FAB) calcd. for $C_7H_{15}N_3O_3NaS$ $(M+Na)^+$: *m/e* 244.0732, found *m/e* 244.0737.

5-azidopentyl methanesulfonate (18). To a solution of dimesylate **15** (12.6 g, 48 mmol) in DMF (100 mL) was added sodium azide (3.14 g, 48 mmol). The reaction mixture was heated to 70 ºC for 90 min, then cooled to room temperature and diluted with Et_2O. The organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and concentrated under vacuum. Flash Chromatography (1:1, CH_2Cl_2/Hexanes) gave 3.12 g (31%) of **18** as colourless oil. R_f = 0.35 (CH_2Cl_2/Hexanes, 7:3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.23 (t, 2H, *J* = 6.5 Hz), 3.29 (t, 2H, *J* = 6.7 Hz), 3.00 (s, 3H), 1.81-1.75 (m, 2H), 1.66-1.60 (m, 2H), 1.53-1.47 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 69.5, 51.1, 37.3, 28.7, 28.2, 22.7; HRMS (FAB) calcd. for $C_6H_{13}N_3O_3NaS$ $(M+Na)^+$: *m/e* 230.0575, found *m/e* 230.0568.

Synthesis of azidoalkyl-1-thioacetates 20-22. General Procedure.

To a solution of the corresponding azidoalkylalcohol **17-19** (100 mol%) in DMF was added potassium thioacetate (110 mol%) dropwise at room temperature. The reaction mixture was stirred overnight, washed with water and extracted tree times

with EtOAc. The combined organic phases were washed with saturated $NaHCO_3$ aqueous solution and brine, dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified by flash chromatography yielding the product.

4-azidobutyl-1-thioacetate (20).

It was prepared following the general procedure from **17** (4.76 g, 24.7 mmol) in DMF (70 mL). Purified by column chromatography using EtOAc/Hexanes, 1/9, affording 3.58 (84% yield) of **20** as a yellow oil; R_f = 0.65 (EtOAc/Hexanes, 1:9); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.35-3.29 (m, 2H), 2.94-2.90 (m, 2H), 2.36 (s, 3H), 1.71-1.66 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.9, 30.6, 28.5, 27.9, 26.8.

5-azidopentyl-1-thioacetate (21).

It was prepared following the general procedure from **18** (2.9 g, 14.1 mmol) in DMF (100 mL). Purification by column chromatography using hexane: EtOAc, 98:2 gave 2.32 g (82% yield) of **21** as a yellow oil; R_f = 0.48 (Hexanes/EtOAc, 9:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.26 (t, 2H, *J* = 6.5 Hz), 2.87 (t, 2H, *J* = 6.7 Hz), 2.32 (s, 3H), 1.62-1.55 (m, 4H), 1.47-1.41 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.7, 51.2, 30.6, 29.1, 28.8, 28.4, 25.8; HRMS (FAB) calcd. for $C_7H_{14}N_3OS$ $(M+H)^+$: *m/e* 188.0858, found *m/e* 188.0858.

6-azidohexyl-1-thioacetate (22).

It was prepared following the general procedure from **19** (23.4 g, 105.7 mmol) in DMF (250 mL). Purification by column chromatography using hexane: EtOAc gave 18.07 g (85% yield) of **22** as a yellow oil; R_f = 0.30 (Hexanes/EtOAc, 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.27 (t, 2H, *J* = 6.8 Hz), 2.86 (t, 2H, *J* = 7.2 Hz), 2.32 (s, 3H), 1.66-1.53 (m, 4H), 1.44-1.35 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.7,

51.2, 30.5, 29.3, 28.8, 28.6, 28.1, 26.1; HRMS (FAB) calcd. for $C_8H_{15}N_3ONaS$ $(M+Na)^+$: *m/e* 224.0834, found *m/e* 224.0831.

Synthesis of azidoalkanesulfinyl chlorides 23-25. General procedure.

To a solution of the adequate thioacetate **20-22** (100 mol%) in methylene chloride at -20ºC, acetic anhydride (100 mol%) and sulfuryl chloride (200 mol %) were added. The resulting mixture was stirred for 1h at -5ºC and then the solvent was evaporated and the residue was dried under vacuum to give the corresponding sulfinyl chloride, **23-25**, that was used without further purification in the following reaction for the preparation of sulfinate esters.

Synthesis of (*R*)-DAG azidoalkanesulfinates. General procedure.

To a solution of 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl (DAGOH) (100 mol %) and pyridine (300 mol %)) in anhydrous toluene, cooled to -78ºC and placed under argon atmosphere, the azidoalkane-1-sulfinyl chloride (200 mol %) was added while the reaction mixture was being vigorously stirred. After stirring at -78ºC for 3h, the reaction mixture was treated with 1M HCl aqueous solution and extracted with CH_2Cl_2. The combined organic layers were successively washed with saturate $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4 and evaporated, to give the *R* sulfinate as the major diastrereomer. The crude product was purified by clumn chromatography obtaining the diastereomerically pure *R* sulfinate.

(*R*)-(1,2:5,6-Di-O-isopropylidene-α-D-glucofuranosyl) 4-azidobutanesulfinate (10-R_S).

It was prepared following the general procedure from DAGOH (1.58 g, 6.065 mmol) and pyridine (1.5 mL) in anhydrous toluene (30 mL), and 4-azidobutyl-1-

sulfinyl chloride (2.2 g, 12.13 mmol). Diastereomeric ratio: **10***R*:**10***S* = 77:23. Purification by column chrotagraphy, hexanes /2-propanol, 20:1 , gave 1.73 mg (70 % yield) of the major diasteromer **10**-*Rs* as yellow oil (90 % of combined yield). R_f = 0.45 (Hexanes/ 2-propanol, 15:1); $[\alpha]_D$= + 5.6 (c = 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 5.86 (d, 1H, *J* = 3.5 Hz), 4.93 (d, 1H. *J* = 3.6 Hz), 4.85 (d, 1H, *J* = 2.9 Hz), 4.39 (dd, 1H, *J* = 2.9 Hz, *J* = 8.7 Hz), 4.26-4.22 (m, 1H), 4.07 (dd, 1H, *J* = 4.6 Hz, *J* = 8.8 Hz), 3.99 (dd, 1H, *J* = 6.1 Hz, *J* = 8.8 Hz), 2.52 (t, 2H, *J* = 6.8 Hz), 2.42-2.29 (m, 2H), 1.41-1.35 (m, 2H), 1.34 (s, 3H), 1.33 (s, 3H), 1.22 (s, 3H), 1.06-1.00 (m, 2H), 0,99 (s, 3H); ^{13}C NMR (125 MHz, C_6D_6) δ 112.7, 109.9, 106.2, 84.8, 83.8, 81.6, 73.1, 68.2, 57.5, 50.9, 28.2, 27.4, 27.2, 26.4, 25.8, 18.9. HRMS *m/e* calcd. for $C_{16}H_{28}N_3O_7S$ $(M+H)^+$: 406.1648, found: 406.1654.

(*R*)-(1,2:5,6-Di-O-isopropylidene-α-D-glucofuranosyl) 5-azidopentanesulfinate (26-R_S).

It was prepared following the general procedure from DAGOH (609 mg, 2.38 mmol) and pyridine (575 μL) in anhydrous toluene (15 mL) and 5-azidopentane-1-sulfinyl chloride **24** (915 mg, 4.68 mmol). The product was obtained as a 83:17 mixture of *R*:*S* diastereomers. Purification by column chromatography, hexane/2-propanol (20:1), gave 717 mg (72% yield) of the major diasteromer **26**-R_S as yellow oil, (87 % of combined yield). R_f = 0.40 (Hexanes/ 2-propanol, 10:1); $[\alpha]_D$ = - 6.02 (c = 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 5.97 (d, 1H, *J* = 3.5 Hz), 5.05 (d, 1H. *J* = 3.5 Hz), 4.97 (d, 1H, *J* = 3.0 Hz), 4.50 (dd, 1H, *J* = 3.0 Hz, *J* = 8.5 Hz), 4.38-4.34 (m, 1H), 4.17 (dd, 1H, *J* = 4.5 Hz, *J* = 8.5 Hz), 4.10 (dd, 1H, *J* = 6.0 Hz, *J* = 8.5 Hz), 2.67 (t, 2H, *J* = 6.5 Hz), 2.58-2.44 (m, 2H), 1.50-1.45 (m, 2H), 1.45 (s, 3H),

1.44 (s, 3H), 1.33 (s, 3H), 1.11-1.03 (m, 2H), 1,09 (s, 3H), 1.05-0.95 (m, 2H); ^{13}C NMR (125 MHz, C_6D_6) δ 112.3, 109.6, 105.9, 84.5, 83.5, 81.3, 72.8, 67.9, 57.7, 50.9, 28.5, 27.1, 26.9, 26.1, 25.9, 25.5, 20.8; HRMS (FAB) calcd. for $C_{17}H_{29}N_3O_7NaS$ $(M+Na)^+$: *m/e* 442.1624, found *m/e* 442.1630.

(*R*)-(1,2:5,6-Di-O-isopropylidene-α-D-glucofuranosyl) 6-azidohexanesulfinate (27-R_S).

It was prepared following the general procedure from DAGOH (7.86 g, 30.2 mmol) and pyridine (8.8 mL) in anhydrous toluene (60 mL), and 6-azidohexane-1-sulfinyl chloride (36 mmol). The product was obtained as a 82:18 mixture of *R*:*S* diastereomers. Purification by column chromatography, hexane/2-propanol (20:1), gave 9.25 g (71 % yield) of the major diasteromer **27**-R_S as yellow oil (86 % of combined yield). R_f = 0.26 (Hexanes/ 2-propanol, 10:1); $[\alpha]_D$ = - 5.2 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90 (d, 1H, *J* = 3.6 Hz), 4.77 (d, 1H, *J* = 3.9 Hz), 4.71 (d, 1H, *J* = 1.9 Hz), 4.16-4.08 (m, 3H), 4.03-3.97 (m, 1H), 3,27 (t, 2H, *J* = 6.74 Hz), 2.91-2.71 (m, 2H), 1.81-1.70 (m, 2H), 1.66-1.55 (m, 2H), 1.49 (s, 3H), 1.48-1.42 (m, 4H), 1.42 (s, 3H), 1.32 (s, 3H), 1,29 (s, 3H, CH_3); ^{13}C NMR (75 MHz, CDCl3) δ 112.4, 109.4, 105.3, 83.8, 83.0, 80.9, 72.2, 67.7, 57.6, 51.3, 28.6, 28.3, 26.8, 26.7, 26.4, 26.2, 25.3, 20.9; HRMS (FAB) calcd. for $C_{18}H_{32}N_3O_7S$ $(M+H)^+$: *m/e* 434.1961, found *m/e* 434.1950.

Synthesis of (*S*)-DAG azidoalkanesulfinates. General procedure.

To a solution of 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl (DAGOH) (100 mol %) and *N*,*N*-diisopropylehtylamine (300 mol %) in anhydrous toluene, cooled to -78ºC and placed under argon atmosphere, the azidobutyl-1-sulfinyl

chloride (200 mol %) was added while the reaction mixture was being vigorously stirred. After stirring at -78ºC for 3h, the reaction mixture was treated with HCl 1M and extracted with CH_2Cl_2. The combined organic layers were successively washed with saturate $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4 and evaporated to give the corresponding sulfinate as a mixture of diastereomers, with the S isomer as the major one. The crude product was purified by silica gel chromatography to give the optically pure *S* sulfinate

(*S*)-(1,2:5,6-Di-O-isopropylidene-α-D-glucofuranosyl) 4-azidobutanesulfinate (10-*Ss*).

Prepared following the general procedure from DAGOH (1.12 g, 4.3 mmol) and *N*,*N*-diisopropylehtylamine (2.25 mL) in anhydrous toluene (20 mL) and 4-azidobutyl-1-sulfinyl chloride **23** (1.56 g, 8.6 mmol). Diastereomeric ratio **10***S*:**10***R*= 92:8. Purification by column chromatography, hexane /2-propanol, 20:1, to give 1.52 g (87 % yield) of the major diasteromer **10**-S_S as yellow oil (91.5 % of combined yield). R_f = 0.40 (Hexanes/ 2-propanol, 15:1); $[\alpha]_D$= - 55.2 (c = 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 5.82 (d, 1H, *J* = 3.6 Hz), 4.83 (d, 1H, *J* = 2.7 Hz), 4.50 (d, 1H , *J* = 3.6 Hz), 4.47 (dd, 1H , *J* = 7.2 Hz, *J* = 2.7 Hz), 4.45-4.41 (m, 1H), 4.15 (dd, 1H, *J* = 8.5 Hz, *J* = 5.2 Hz), 4.09 (dd, 1H, *J* = 8.4 Hz, *J* = 6. 1 Hz), 2,51 (t, 2H, *J* = 6.75 Hz), 2.22 (t, 2H, *J* = 7.8 Hz), 1.40 (s, 3H), 1.35 (s, 3H), 1.34-1.25 (m, 2H), 1.30 (s, 3H), 1.06 (s, 3H), 1.05-0.97 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 112.7, 109.8, 105.9, 84.6, 81.3, 79.5, 73.3, 67.6, 56.8, 50.9, 28.3, 27.4, 27.2, 26.6, 25.8, 19.0.

(*S*)-(1,2:5,6-Di-O-isopropylidene-α-D-glucofuranosyl) 5-azidopentanesulfinate (26-S_S).

Prepared following the general procedure from DAGOH (605 mg, 2.32 mmol) and *N*,*N*-diisopropylehtylamine (1.2 mL) in anhydrous toluene (15 mL) and 5-azidopentane-1-sulfinyl chloride **24** (910 mg, 4.64 mmol). The sulfinate was obtaines as a 94:6 (**24***S*: **24***R*) mixture of diastereomers. Purification by column chromatography, hexane / 2-propanol, 20:1, gave 870 mg (89.3 % yield) of the major diasteromer **26**-S_S as yellow oil (92 % of combined yield). R_f = 0.42 (Hexanes/ 2-propanol, 10:1); $[\alpha]_D$= - 49.95 (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 5.82 (d, 1H, *J* = 3.5 Hz), 4.85 (d, 1H, *J* = 2.5 Hz), 4.51 (d, 1H , *J* = 3.5 Hz), 4.49-4.43 (m, 2H), 4.16 (dd, 1H, *J* = 8.5 Hz, *J* = 5.5 Hz), 4.10 (dd, 1H, *J* = 8.5 Hz, *J* = 6.0 Hz), 2,57 (t, 2H, *J* = 6.5 Hz), 2,29 (t, 2H, *J* = 7.5 Hz), 1.41 (s, 3H), 1.35 (s, 3H), 1.34-1.26 (m, 2H), 1.29 (s, 3H), 1.07 (s, 3H), 1.00-0.96 (m, 2H), 0.93-0.87 (m, 2H); ^{13}C NMR (125 MHz, C_6D_6) δ 112.4, 109.5, 105.6, 84.3, 81.0, 79.1, 73.0, 67.3, 57.0, 50.9, 28.5, 27.0, 26.9, 26.3, 25.9, 25.5, 20.9; HRMS (FAB) calcd. for $C_{17}H_{29}N_3O_7NaS$ $(M+Na)^+$: *m/e* 442.1624, found *m/e* 442.1630.

(*S*)-(1,2:5,6-Di-O-isopropylidene-α-D-glucofuranosyl) 6-azidohexanesulfinate (27-S_S).

Prepared following the general procedure from DAGOH (7.32 g, 28 mmol) and *N*,*N*-diisopropylehtylamine (15 mL) in anhydrous toluene and 6-azidohexane-1-sulfinyl chloride **25** (33.7 mmol). The product was obtained as a 92:8 mixture of *S*:*R* diastereomers . Purification by column chromatography, hexane/2-propanol (20:1), gave 9.53 g (78.4 % yield) of the major diastereoisomer **27**-S_S as yellow oil (85.2%

of combined yield). R_f = 0.25 (Hexanes/ 2-propanol, 10:1); $[\alpha]_D$= - 39.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90 (d, 1H, *J* = 3.6 Hz), 4.74 (d, 1H, *J* = 2.5 Hz), 4.60 (d, 1H , *J* = 3.70 Hz), 4.32-4.24 (m, 2H), 4.09 (dd, 1H, *J* = 8.5 Hz, *J* = 5.8 Hz), 4.01 (dd, 1H, *J* = 8.5 Hz, *J* = 5.0 Hz), 3,27 (t, 2H, *J* = 6.74 Hz), 2.88-2.70 (m, 2H), 1.78-1.67 (m, 2H), 1.66-1.56 (m, 2H), 1.51 (s, 3H), 1.44-1.36 (m, 4H), 1.43 (s, 3H), 1.34 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 112.4, 109.2, 104.9, 83.6, 80.3, 79.2, 72.3, 66.7, 57.1, 51.2, 28.5, 28.3, 26.7, 26.7, 26.3, 26.2, 25.2, 21.1; HRMS (FAB) calcd. for $C_{18}H_{31}N_3O_7NaS$ $(M+Na)^+$: *m/e* 456.1780, found *m/e* 456.1780.

Synthesis of (*S*)- and (*R*)-azidoalkyl sulfoxides. General procedure.

To a solution of the (*R*)- or (*S*)-DAG azidoalkanesulfinate ester (100 mol%) in anhydrous toluene, at 0ºC, the adequate alkyl magnesium bromide solution (150 mol %) was added. After stirring for 1h at 0ºC, saturated NH_4Cl aqueous solution was added. The aqueous layer was extracted with CH_2Cl_2 and the resulting organic layers were combined, dried on Na_2SO_4, and concentrated. The crude product was purified by silica gel chromatography.

(*S*)-(-)-1-Azido-4-(methylsulfinyl)-butane (28-S_S).

Prepared following the general procedure from sulfinate ester **10**-R_S (1.41 g, 3.48 mmol) in anhydrous toluene (20 mL), and methyl magnesium bromide 1.4M (3.75 ml, 5.22 mmol). Purification by column chromatography EtOC/MeOH (9:1) gave 486 mg (87% yield) of the compound **27**-S_S as a colourless liquid. R_f = 0.31 (EtOAc / MeOH, 9:1); $[\alpha]_D$ = + 86,33 (c = 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.34 (dt, 2H, *J* = 2.0 Hz, *J* = 6.6 Hz), 2.76-2.64 (m, 2H), 2.56 (s, 3H), 1.89-1.83 (m, 2H),

1.81-1.68 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.8, 50.8, 38.4, 28.0, 20.0; HRMS *m/e* calcd. for $C_5H_{12}N_3OS$ $(M+H)^+$: 162.0701, found: 162.0699.

(*R*)-(-)-1-Azido-5-(methylsulfinyl)-pentane (29-R_S).

Prepared following the general procedure from sulfinate ester **26**-S_S (440 mg, 1.05 mmol) in anhydrous toluene (10 mL), and methyl magnesium bromide 1.4M (1.12 ml, 1.57 mmol). Purification by column chromatography, EtOAc/ MeOH (9:1), to give 135 mg (74% yield) of sulfoxide **29**-R_S as a colourless liquid. R_f = 0.16 (EtOAc / MeOH, 9:1); $[\alpha]_D$ = - 60,42 (c = 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.29 (t, 2H, *J* = 6.5 Hz), 2.79-2.73 (m, 1H), 2.69-2.63 (m, 1H), 2.58 (s, 3H), 1.84-1.77 (m, 2H), 1.67-1.59 (m, 2H), 1.57-1.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 54.2, 51.0, 38.4, 28.5, 25.9, 22.2; HRMS (FAB) *m/e* calcd. for $C_6H_{13}N_3ONaS$ $(M+Na)^+$: 198.0677, found: 198.0680.

(*S*)-(-)-1-Azido-5-(methylsulfinyl)-pentane (29-S_S).

Prepared following the general procedure from sulfinate ester **26**-R_S (387 mg, 0.92 mmol) in anhydrous toluene (15 mL), and methyl magnesium bromide 1.4M (1.0 ml, 1.38 mmol). Purification by column chromatography, EtOAc/ MeOH (9:1) gave 125 mg (78% yield) of sulfoxide **29**-S_S as a yellow liquid, with identical spectroscopic characteristics than the **29**-R_S enantiomer. $[\alpha]_D$ = + 61.25 (c = 0.8, $CHCl_3$); HRMS (FAB) *m/e* calcd. for $C_6H_{13}N_3ONaS$ $(M+Na)^+$: 198.0677, found: 198.0670.

(*R*)-(-)-1-Azido-6-(methylsulfinyl)-hexane (30-R_S).

Prepared following the general procedure from sulfinate **26**-S_S (2.89 g, 6.66 mmol) in anhydrous toluene (15 mL), and methyl magnesium bromide 1.4M (7.2 ml, 10 mmol). Purification by column chromatography, EtOAc/ MeOH (9:1) gave

926 mg (73% yield) of sulfoxide **30**-R_S as a colourless liquid. R_f = 0.17 (EtOAc / MeOH, 9:1); $[\alpha]_D$ = - 64,24 (c = 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.26 (t, 2H, *J* = 6.9 Hz), 2.77-2.58 (m, 2H), 2.54 (s, 3H), 1.82-1.72 (m, 2H), 1.65-1.36 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 54.5, 51.2, 38.6, 28.5, 28.3, 26.3, 22.4; HRMS (FAB) *m/e* calcd. for $C_7H_{16}N_3OS$ $(M+H)^+$: 190.1014, found: 190.1021.

(*S*)-(-)-1-Azido-6-(methylsulfinyl)-hexane (30-S_S).

Prepared following the general procedure from sulfinate ester **27**-R_S (2.55 g, 5.87 mmol) in anhydrous toluene (50 mL), and methyl magnesium bromide 1.4M (6.3 ml, 8.8 mmol). Purification by column chromatography, EtOAc/ MeOH (9:1) gave 895 mg (81% yield) of sulfoxide **30**-S_S as a colourless liquid, with identical spectroscopic characteristics than the **30**-R_S enantiomer. $[\alpha]_D$ = + 61,00 (c = 0.6, $CHCl_3$); HRMS (FAB) *m/e* calcd. for $C_7H_{15}N_3ONaS$ $(M+Na)^+$: 212.0834, found: 212.0836.

(*R*)-(-)-1-Azido-4-(ethylsulfinyl)-butane (31-R_S).

Prepared following the general procedure from sulfinate ester **10**-S_S (320 mg, 0.78 mmol) in anhydrous toluene (10 mL), and ethyl magnesium bromide 1.0 M (1.2 ml, 1.2 mmol). Purification by column chromatography, EtOAc/ MeOH (9:1) gave 122 mg (90% yield) of sulfoxide **31**-R_S as a colourless liquid. R_f = 0.35 (EtOAc / MeOH, 9:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.34 (dt, 2H, J_1 = 1.8 Hz, J_2 = 6.5 Hz), 2.76-2.69 (m, 3H), 2.68-2.612 (m, 2H), 1.92-1.83 (m, 2H), 1.82-1.69 (m, 2H), 1.33 (t, 3H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.9, 50.8, 45.6, 28.0, 20.1, 6.8; HRMS *m/e* calcd. for $C_6H_{14}N_3OS$ $(M+H)^+$: 176.0858, found: 176.0863.

(*R*)-(-)-1-Azido-4-(butylsulfinyl)-butane (32-R_S).

Prepared following the general procedure from sulfinate ester **10**-S_S (334 mg, 0.82 mmol) in anhydrous toluene (50 mL), and butyl magnesium bromide 2.0 M (0.6 ml, 1.23 mmol). Purification by column chromatography, EtOAc/ MeOH (9:1) gave 158 mg (95% yield) of sulfoxide **32**-R_S as a colourless liquid. R_f = 0.68 (EtOAc / MeOH, 9:1); $[\alpha]_D$ = - 9,18 (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.35 (dt, 2H, *J* = 2.2 Hz, *J* = 6.6 Hz), 2.79-2.72 (m, 2H), 2.70-2.62 (m, 2H), 1.92-1.86 (m, 2H), 1.82-1.70 (m, 4H), 1.55-1.42 (m, 2H), 0.96 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.1, 51.5, 50.9, 28.0, 24.6, 22.0, 20.1, 13.6; HRMS *m/e* calcd. for $C_8H_{18}N_3OS$ $(M+H)^+$: 204.1171, found: 204.1165.

Synthesis of (*R*)- and (*S*)-isothiocyanates. General Procedure.

To a solution of the corresponding azide derivative, **28-32,** (100 mol %) in Et_2O was added triphenylphosphine (190 mol %), and the reaction was refluxed for 1h.. After removing the solvent at vacuum, carbon disulfide was added and the mixture was refluxed for 3h. Finally the solvent was removed under vacuum and the crude product was purified by silica gel chromatography.

(-)-(*R*)-1-isothiocyanate-4-(butylsulfinyl)-butane (2-R_S).

It was prepared following the general procedure from the azide **28**-R_S (140 mg, 0.69 mmol) in Et_2O (5mL), triphenylphosphine (343 mg, 1.31 mmol), and carbon disulfide (3 mL). ; Purification by column chromatography, EtOAc/ MeOH (9:1), affording 149 mg (98% yield) of **2***R* as colourless liquid. R_f = 0.15 (EtOAc / MeOH, 9:1); $[\alpha]_{D\,=}$ -80.81 (c = 1.0 , $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.59 (t, 2H, *J* = 6.0Hz), 2.76-2.62 (m, 4H), 1.98-1.82 (m, 4H), 1.79-1.72 (m, 2H), 1.56-1.43 (m, 2H), 0.98 (t, 3H, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.4, 51.3, 44.7, 29.1, 24.6,

22.1, 20.2, 13.7; HRMS *m/e* calcd. for $C_9H_{17}NONaS_2$ $(M+Na)^+$: 242.0649, found: 242.0654.

(+)-(*S*)-1-isothiocyanate-4-(methylsulfinyl)-butane (2-S_S).

It was prepared following the general procedure fron azide **28**-S_s (248 mg, 1.55 mmol) in Et_2O (15 mL), triphenylphosphine (770 mg, 2.93 mmol), and carbon disulfide (5 mL). Purification by column chromatography, EtOAc/ MeOH (9:1), gave 215 mg (78 % yield) of **2**$S_{S,}$ as a colourless liquid. R_f = 0.30 (EtOAc / MeOH, 9:1); $[\alpha]_{D\,=}$ + 80.00 (c = 0.8, $CHCl_3$); Spectroscopical data identical to those of the **2-*R*$_S$** enantiomer. HRMS *m/e* calcd. for $C_6H_{12}NOS_2$ $(M+H)^+$: 178.0360, found: 178.0358.

(-)-(*R*)-1-isothiocyanate-5-(methylsulfinyl)-pentane (4-R_S).

It was prepared following the general procedure from the azide derivative **29**-R_S (115 mg, 0.66 mmol), triphenylphosphine (327 mg, 1.25 mmol) in anhydrous Et_2O (3 mL), and carbon disulfide (1 mL). Purification by column chromatography, EtOAc/ MeOH (9:1), gave 106 mg (91% yield) of **4**-R_S, as an ambar syrup. R_f = 0.25 (EtOAc / MeOH, 9:1); $[\alpha]_{D\,=}$ - 73.48 (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.54 (t, 2H, *J* = 6.5 Hz), 2.76-2.63 (m, 2H), 2.57 (s, 3H), 1.86-1.73 (m, 4H), 1.67-1.55 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 54.2, 44.7, 38.7, 29.6, 25.8, 21.9; HRMS *m/e* calcd. for C7H13NONaS$_2$ $(M+Na)^+$: 214.0336, found: 214.0339.

(+)-(*S*)-1-isothiocyanate-5-(methylsulfinyl)-pentane (4-S_S).

It was prepared following the general procedure from the azide derivative **28**-S_S (99.6 mg, 0.57 mmol), triphenylphosphine (283 mg, 1.08 mmol) in anhydrous Et_2O (3 mL) and carbon disulfide (1 mL). Purification by column chromatography, EtOAc/ MeOH (9:1), gave 106 mg (97% yield) of **4**-S_S, as anambar syrup. The

spectroscopic data are identical to those of its enantiomer, **4-*R*S**. $[\alpha]_{D\ =}$ + 69.23 (c = 0.8, $CHCl_3$); HRMS *m/e* calcd. for C7H14NOS$_2$ $(M+H)^+$: 192.0517, found: 192.0515.

(*R*)-(-)-1-isothiocyanato-6-(methylsulfinyl)hexane (5-*R*S).

It was prepared following the general procedure fro the azide derivative **30**-*R*S (816 mg, 4.32 mmol), triphenylphosphine (2.15 g, 8.2 mmol) in anhydrous Et_2O (3 mL) and carbon disulfide (6.5 mL). Purification by column chromatography, EtOAc/ MeOH (9:1), gave 710 mg (81% yield) of **5**-*R*S, as an ambar syrup. R_f = 0.4 (EtOAc / MeOH, 9:1); $[\alpha]_{D\ =}$ - 70.62 (c = 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.52 (t, 2H, *J* = 10.5 Hz), 2.77-2.60 (m, 2H), 2.56 (s, 3H), 1.83-1.67 (m, 4H), 1.53-1.46 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 54.4, 44.9, 38.7, 29.6, 27.9, 26.2, 22.4; HRMS *m/e* calcd. for C8H16NOS$_2$ $(M+H)^+$: 206.0673, found: 206.0669.

(+)-(*S*)-1-isothiocyanate-6-(methylsulfinyl)-hexane (5-*S*S).

It was prepared following the general procedure, from the azido derivative **30**-*S*S (870 mg, 4.6 mmol), triphenylphosphine (2.3 g, 8.75 mmol) in anhydrous Et_2O (3 mL), and carbon disulfide (7 mL). Purification by column chromatography, EtOAc/ MeOH (9:1), gave 820 mg (87% yield) of **5**-*S*S, as an ambar syrup. The spectroscopic data are identical to those of its enantiomer, **5**-*R*S. $[\alpha]_{D\ =}$ + 68.77 (c = 1.0, $CHCl_3$); HRMS *m/e* calcd. for C8H16NOS$_2$ $(M+H)^+$: 206.0673, found: 206.0677.

(*R*)-(-)1-isothiocyanato-4-(ethylsulfinyl)-butane (6-*R*S).

It was prepared following the general procedure from the azide derivative **31**-*R*s (90 mg, 0.51 mmol) in Et_2O (4 mL), triphenylphosphine (261 mg, 0.99 mmol), and carbon disulfide (1 mL). Purification by column chromatography, EtOAc/ MeOH

(9:1), gave 92 mg (95% yield) of **6-*R*$_S$** as a yellow liquid. R_f = 0.20 (EtOAc / MeOH, 9:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.59 (t, 2H, *J* = 5.9Hz), 2.78-2.70 (m, 3H), 2.70-2.63 (m, 1H), 1.99-1.82 (m, 4H), 1.35 (t, 3H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.6, 46.0, 44.7, 29.1, 20.2, 6.8; HRMS *m/e* calcd. for $C_7H_{13}NONaS_2$ $(M+Na)^+$: 214.0336, found: 214.0338.

(-)-(*R*)-1-isothiocyanato-4-(butylsulfinyl)-butane (7-*R*$_S$).

It was prepared following the general procedure from the azide derivative **32-*R*$_S$** (140 mg, 0.69 mmol) in Et_2O (5 mL), triphenylphosphine (343 mg, 1.31 mmol), and carbon disulfide (3 mL). Purification by column chromatography, EtOAc/MeOH (9:1), gave 149 mg (98% yield) of **7-*R*$_S$** as a colourless liquid. R_f = 0.15 (EtOAc / MeOH, 9:1); $[\alpha]_D$ = -8.81 (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.59 (t, 2H, *J* = 6.0Hz), 2.76-2.62 (m, 4H), 1.98-1.82 (m, 4H,), 1.79-1.72 (m, 2H), 1.56-1.43 (m, 2H), 0.98 (t, 3H, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.4, 51.3, 44.7, 29.1, 24.6, 22.1, 20.2, 13.7; HRMS *m/e* calcd. for $C_9H_{17}NONaS_2$ $(M+Na)^+$: 242.0649, found: 242.0654.

4.2. Biological activity of sulforaphane and sulforaphane analogues

4.2.1. Activation of Nrf2

- *Construction of a rNQO1-ARE luciferase reporter plasmid*

The rat NQO1-ARE luciferase reporter plasmid (pGL3-rNQO1 ARE) has previously been described [63].

- *Transient transfection and luciferase reporter assays.*

HaCaT cells (human immortalized keratinocyte cell line) were cultured in DMEM medium 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 x 10^5 cells/well in 12-well plates (Nunc) in 1ml DMEM, 10% FCS, 1% Penicillin/Streptomycin and grown until 80% (24h). Cells were washed twice with PBS, placed in 1ml Opti-MEM I (GIBCO) and co-transfected with 2µg of the rNQO1-ARE luciferase reporter plasmid and 0.01µg of the phRL-CMV *Renilla* luciferase vector plasmid (Promega) as an internal control for transfection efficiency. Both plasmid DNAs were diluted with 106µl 0.9% (w/v) sodium chloride solution. 3.8µl of polyethylenimine solution (1mg/ml) was then added and briefly vortexed. The mixture was incubated at room temperature for 15min to allow complex formation. Afterwards the transfection mix was added to the cells. After 4h the transfection mix was fully replaced by fresh cell culture medium (DMEM, 10% FCS, 1% Penicillin/Streptomycin). After 24h the cells were washed with PBS and incubated with fresh culture medium containing different concentrations of the sulforaphane homologues (50nm, 0.5µM, 5µM, 50µM, 500µM) or DMSO as negative control. Cells were harvested 20h later using Passive Lysis Buffer (Promega). The firefly and *renilla* luciferase activities were measured in the supernatants using the Dual-Luciferase Reporter Assay System kit from Promega according to the manufacture's instructions. Luciferase activity was determined on a MicroLumatPlus LB96V (EG&G Berthold) and reported as x-fold activation compared to DMSO-treated cells. Results show mean plus/minus S.D. from triplicate determinations.

4.2.2. CYTOTOXIC ACTIVITY

All cell lines used (A-549, MRC5) were obtained from the European collection of cell cultures (European Collection of Cell Cultures). Both were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 2 mM glutamine, 50 μg / mL of both penicillin and streptomycin and 10% fetal bovine serum (FBS) and have been incubated in an atmosphere of air at 95 % humidity, 5% CO_2 and 37 °C.

The cellular proliferation/viability was assessed by the MTT (3 – [4,5 - dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The MRC-5 cell line has been cultivated at $9x10^3$ cells / well, while A-459 cells were cultivated at 4.5 $x10^3$ cellsd/ well, in a 96 well plate for 24h. After this time, sulforaphane and sulforaphane homologues were added to the wells. After 48h the cell medium was removed and 125 μl of MTT (1 mg / ml in medium) was added and allowed to react for 4h. Then, 80 μl of a 20 % SDS solution in hydrochloric acid (0.002M) was added and the mixture and incubated for 10h at 37 °C. The cell viability was quantified by optical density at 540nm using a multi-well plate reader spectrophotometer. The results are expressed as the percentage of cell viability relative to controls. All data are the result of at least three independent experiments; the results of statistical analysis (*p* value) for the t-test are also indicated.

Acknowledgements

This work was supported by the Ministerio de Economía y Competitividad (grant No. CTQ2010-21755-CO2-02), the Junta de Andalucía (P11-FQM-8046), and Evgen Pharma. We gratefully thank CITIUS for NMR facilities.

References

[1] (a) B. Demmig-Adams, W.W. Adams, *Science.* 2002, **298**, 2149. (b) A. P. Simopoulos, *Biomedicine and Phamacotherapy.* 2002, **56**, 365.

[2] I. Golberg, in "Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals", I. Goldberg, Ed.; 1994; Springer.

[3] M. Fenech, A. El-Sohemy, L. Cahill, L. R. Ferguson, T. A. French, E. S. Tai, J. Milner, W. P. Koh, L. Xie, M. Zucker, M. Buckley, L. Cosgrove, T. Lockett, K. Y. Fung, R. Head, *J. Nutrigenet Nutrigenomics,* 2011, **4**, 69

[4] I. Herr, M. W. Büchler, *Cancer Treat. Rev.* 2010, **36**, 377.

[5] J. W. Lampe, S. Peterson, *J. Nutr.* 2002**, 132**, 2991.

[6] C. C. Conaway, Y. M. Yang, *Curr. Drug Metab.* 2002, **3**, 233.

[7] U. Wittstoc, B. A. Halkier, *Trends Plant Sci.* 2002, **7**, 263.

[8] J. D. Clarke, A. Hsu, K. Riedl, D. Bella, S. J. Schwartz, J. F. Stevens, E. Ho, *Pharmacol. Res*. 2011, **64**, 456.

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

[10] Y. Zhang, P. Talay, C. G. Cho, G. H. Posner, *Proc. Natl. Acad. Sci. USA* 1992, **89**, 2399.

[11] N. Juge, R. F. Mithen, M. Traka, M. *Cell. Mol. Life. Sci.* 2007, **64**, 1105.

[12] F. L. Chung, C. C. Conaway, C. V. Rao, B. S. Reddy, *Carcinogenesis* 2000, **21**, 2287.

[13] B. S. Comblatt, L. Ye, A. T. Dikova-Kostova, M. Erb, J. W. Fahey, N. K Singh, M.-S. Chen, T. Stierer, E. Garret-Mayer, P. Argani, N. E. Davidson, P. Talay, T. Kensler, K. Visvanathan, *Carcinogenesis* 2007, **28**, 1485.

[14] W. S. Xu, W. B. Parmigiani, P. A. Marks, *Oncogene*, 2007, *26*, 5541.

[15] J. K. Min, H. K. So, L. Soo-Jeong, *Anticancer Res.*, 2010, **30**, 3611.

[16] Choi, W. *Biomedicine & Pharmacotherapy*, 2008, **62**, 637.

[17] S. V. Singh, R. Warin, D. Xiao, A. A. Powolny, S. D. Stan, J. A. Arlotti, Y. Zeng, E. Hahm, S. W. Marynowski, A. Bommareddy, D. Desai, S. Amin, R. A. Parise, J. H. Beumer, W. H. Chambers, *Cancer Res*, 2009, **69**, 2117.

[18] N. Hanlon, N. Coldham, M. J. Sauer, C. Ioannides, *Chemico-Biological Interactions*, 2009, **177**, 115.

[19] S. –H. Kim; S. V. Singh. *Mol Cancer Ther.* 2009, **8**, 1946.

[20] J. H. Lee, T. O. Khor, L. Shu, Z.-Y. Su, F. Fuentes, A. –N. T. Kong, *Phramacol. Therap.* 2013, **137**, 153.

[21] J. D. Clarke, A. Hsu, Z. Yu, R. H. Dashwood, E. Ho, *Mol. Nutr. Food Res.* 2011, **55**, 999.

[22] S. M. Meeran, S. N. Patel, T. O. Tollefsbol, *PLoS One*, 2010, **5**, e1145.

[23] A. Hsu, C. P. Wong, Z. Yu, D. E. Williams, R. H. Dashwood, E. Ho, *Clinical Epigenetics* 2011, **3**, 1.

[24] B. A. Halkier, J. Gershenzon, *Ann. Rev. Plant Biol.* 2006, **57**, 303.

[25] A. M. Kore, E. H. Jeffery, M. A. Wallig, *Food Chem. Toxicol.* 1993, **31**, 723.

[26] Y. Morimitsu, Y. Nakagawa, K. Hayashi, H. Fujii, T. Kumagai, Y. Nakaruma, T. Osawa, F. Horio, K. Itoh, K. Iida, M. Yamamoto, K. Uchida, *J. Biol. Chem.* 2002, **277**, 3456.

[27] P. Rose, K. Faulkner, G. Williamson, R. Mithen, *Carcinogenesis,* 2000, 21, 1983.

[28] Y. Zhang, T. W. Kensler, C.-G. Cho, G. H. Posner, P. Talalay, *Proc. Natl. Acad. Sci. USA* 1994, **91**, 3147,

[29] G. H. Posner, C. –G. Cho, J. V. Green, Y. Zhang, P. Talalay, *J. Med. Chem.* 1994, **37**, 170,

[30] J. R. Mays, R. L. W. Roska, S. Sarfaraz, H. mukhtar, S. C. Rajski, *ChemBioChem* 2008, **9**, 729.

[31] K. Hu, Y.-J. Qi, J. Zhao, H. –F. Jiang, X. Chen, J. Ren, *Eur. J. Med. Chem.* 2013, **64**, 529.

[32] P. Kiełbasiński, J. Łuczak, T. Cierpiał, J. Błaszczyk, L. Sieroń, K. Wiktorska, K. Lubelska, M. Milczarek, Z. Chilmończyk, *Eur. J. Med. Chem.* 76 (2014), 332-342

[33] Y. –H. Ahn, Y. Hwang, H. Liu, X. J. Wang, Y. Zhang, K. K. Stephenson, T. N. Boronina, R. N. Cole, A. T. Dinkova-Kostova, P. Talalay, P. A. Cole, *Proc. Natl. Acad. Sci. USA* 2010, **107**, 9590.

[34] F. Vergara, M. Wenzler, B. G. Hansen, D. J. Kliebenstein, B. A. Halkier, J. Gershenzon, B. Schneider, *Phytochemistry* 2008, **69**, 2373.

[35] B. Hansen, D. J. Kliebenstein, B. A. Halkier, *Plant J.* 2007, **50**, 902.

[36] Y. Zhang, L. Tang, *Acta Pharmacol. Sin.* 2007, **28**, 1343.

[37] A. F. A. Razis, R. Iori, C. Ioannides, *Int. J.Cancer*, 2011, **128**, 2775.

[38] A. A. A. Razis, M. Bagatta, G. R. De Nicola, R. Iori, C. Ioannides, *Arch. Toxicol*, 2011, **85**, 919.

[39] N. Khiar, S. Werner, A. Mallouk, F. Lieder, A. Alcudia, I. Fernández, *J. Org. Chem*. 2009, **74**, 6002.

[40] I. Fernández, N. Khiar, *Chem. Rev*. 2003, **103**, 3651,

[41] E. Wojaczynska, J. Wojaczynski, *Chem. Rev.* 2010, **110**, 4303.

[42] R. Bentley, *Chem. Soc. Rev.* 2005, **34**, 609.

[43] E. Carlsson, P. Lindberg, S. von Unge, S. *Chemistry in Britain*. 2002, 42.

[44] J. Cao, T. E. Prisinzano, O. M. Okunola, T. Kopajtic, M. Shook, J. L. Katz, A. M. Newman, *ACS Med. Chem. Lett.* 2011, **2**, 48.

[45] R. Maguire, S. Papot, A. Ford, S. Touhey, R. O'Connor, M. Clynes, *Synlett*, 2001, 41.

[46] F. Rebiere, O. Samuel, L. Ricard, H. B. Kagan, *J. Org. Chem*. **1991**, *56*, 5991.

[47] M. A. M. Capozzi, C. Cardellicchio, F. Naso, V. Rosito. *J. Org. Chem.* **2002**, *67*, 7289.

[48] J. L. García Ruano, C. Alemparte, M. T. Aranda, M. M. Zarzuelo, *Org. Lett.* **2003,** *5*, 75.

[49] Z. Han, D. Krishnamurthy, P. Grover, H. S. Wilkinson, Q. K. Fang, X. Su, Z.-H. Lu, D. Magiera, C. H. Senanayake, *Angew. Chem. Int. Ed*. **2003**, *42*, 2032.

[50] J. Legros, J. R. Delhi, C. Bolm, *Adv. Synth. Catal.* 2005**, 347**, 19.

[51] H. L. Holland, F. M. Brown, B. Larsen, M. Zabic, *Tetrahedron: Asymmetry* 1 995, **6**, 1569.

[52] J. K. Whitesell, M. –S. Wong, *J. Org. Chem.* 1994, **59**, 597.

[53] W. A. Schenk, M. Dürr, *Chem. Eur. J.* 1997, **3**, 713.

[54] I. Fernández, N. Khiar, J. M. Llera, F. Alcudia, *J. Org. Chem.* 1992, **57**, 6789.

[55] D. Balcells, G. Ujaque, I. Fernández, N. Khiar, F. Maseras, *Adv. Synth. Catal.* 2007, **249**, 2103.

[56] N. Khiar, F. Alcudia, J. –L. Espartero, L. Rodríguez, I. Fernández, *J. Am. Chem. Soc.* 2000, **122**, 7598.

[57] W. P. M. Lowik, R. M. J. Liskamp, *Eur. J. Org. Chem.* 2000, 1219.

[58] X. Gao P. Talalay *Proc Natl Acad Sci* U S A. 2004, **101**, 10446.

[59] J. D. Hayes, M. McMahon, *Trends Biochem. Sci.* 2009, **34**, 176.

[60] T. Nguyen P. J. Sherratt, C. B. Pickett, *Annu. Rev. Pharmacol. Toxicol.* 2003**,** **43**, 233.

[61] (a) K. I. Tong, A. Kobayashi, F. Katsuoka, M. Yamamoto, *Biol. Chem.* 2006, **387**, 1311, (b) A. J. Wilson, J. K. Kerns, J. F. Callahan, C. J. Moody, J. *Med. Chem.* 2013, **56**, 7463.

[62] L. V. Favreau. C. B. Pickett, *J. Biol. Chem.* 1995, **270**, 24468.

[63] F. Lieder, F. Reisen, T. Geppert, G. Sollberger, H. –D. Beer, U. auf dem Keller, M. Shäfer, M. Detmar, G. Schneider, S. Werner, *J. Biol. Chem.* 2012, **287**, 33001.

[64] T. Mosmann, T. *J. of Immunological Methods.* 1983, **65**, 55.

[65] F. Denizot, R. Lang, *J. of Immunological Methods*. 1986, **89**, 271.

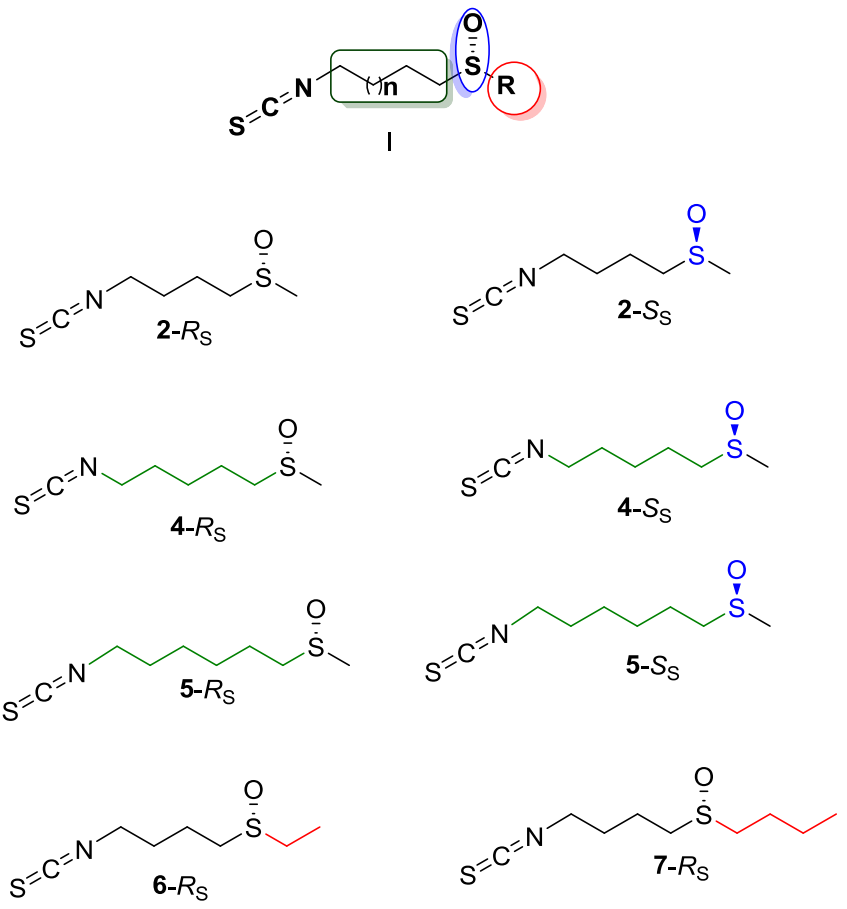


Figure 1: Structure of sulforaphane and homologues synthesized in this work.

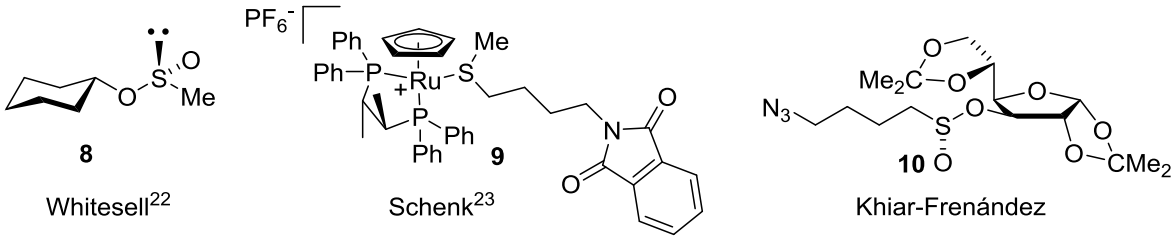


Figure 2: Diastereomeric intermediates used for the synthesis of enantiopure and scalemic R_S-sulforaphane.

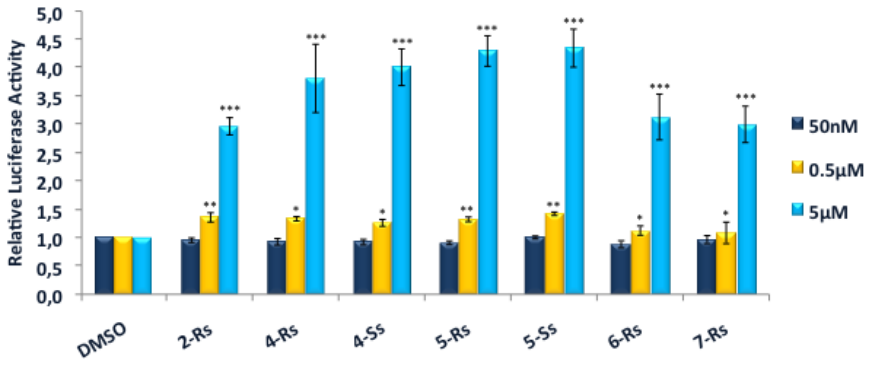


Figure 3: Dose dependent effects of sulforaphane analogues on the activation of an ARE reporter gene. HaCaT keratinocytes were transiently transfected with the rNQO1-ARE luciferase reporter plasmid and the phRL-CMV *Renilla* luciferase vector. Transfected cells were incubated for 24h with different concentrations of the sulforaphane **2**-R_S and homologues **4**-R_S**,** **4**-S_S **5**-R_S**,** **5**-S_S, **6**-R_S**,** and **7**-R_S**.** Results were normalized to the *renilla* luciferase activity. Values show mean +/- S.D. from triplicate determinations. The result was reproduced in an independent transfection experiment.

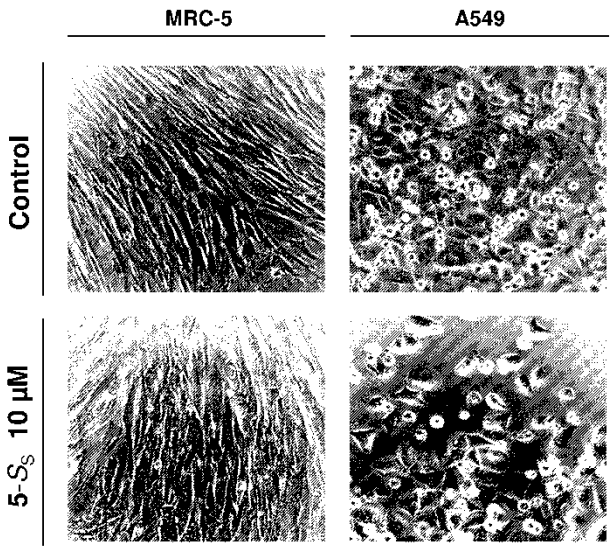
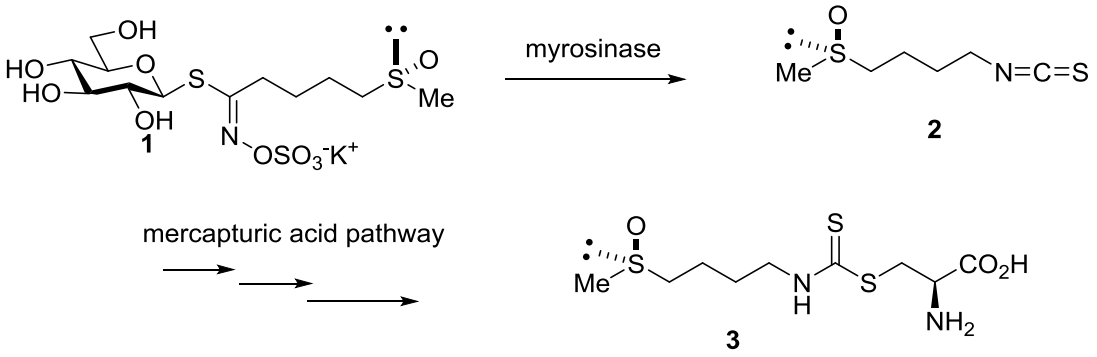
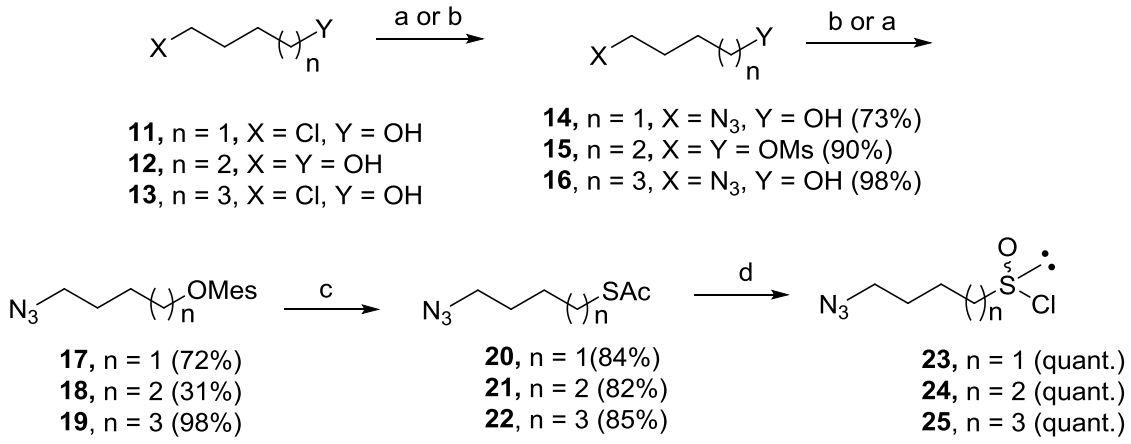


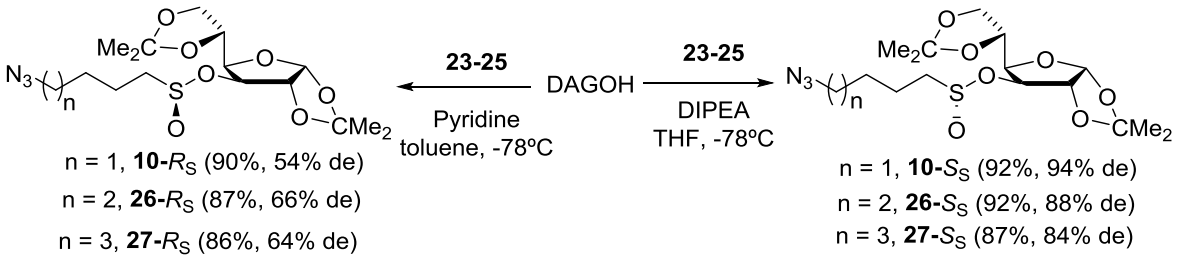
Figure 4. Inverted microscope images corresponding MRC-5 and A549 cell lines following treatment with **5**-S_S analogue at a 10 μM concentration .



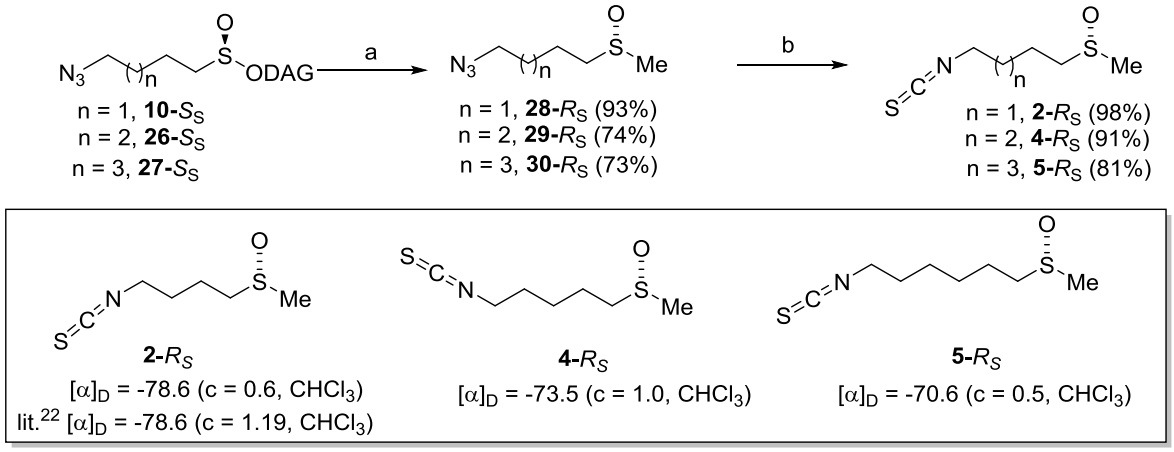
Scheme 1: Biosynthetic pathway to (R_S)-sulforaphane **2** and (R_S, S_C)-sulforaphane-cysteine analogue **3**: Lossen rearrangement through myrosinase deglycosylation of glycopharin **1** followed by mercapturic acid pathway.



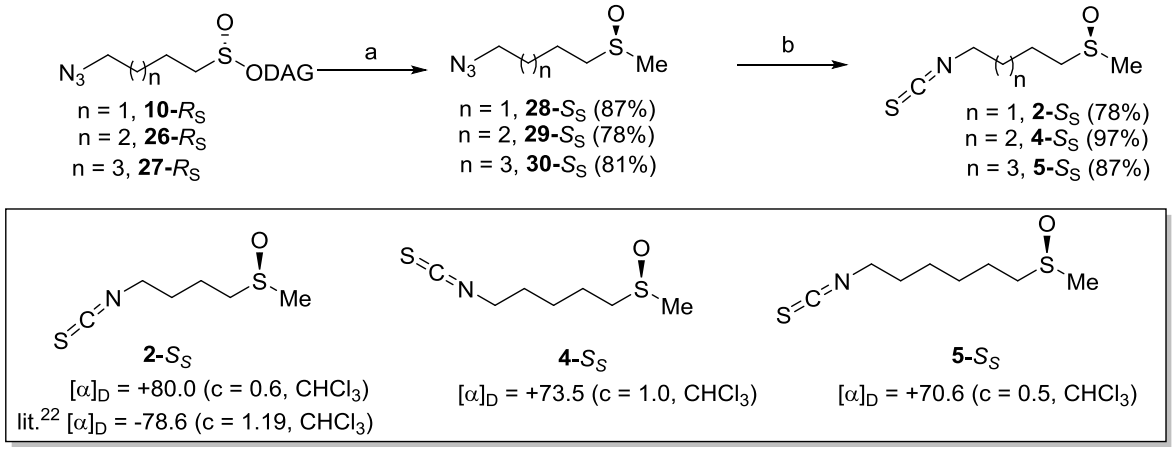
Scheme 2: Synthesis of 1-azido-butanesulfinyl chlorides **23-25**. a) NaN_3, DMF, b) MsCl, NEt_3, CH_2Cl_2, c) KSAc, DMF, d) SO_2Cl_2, Ac_2O, CH_2Cl_2



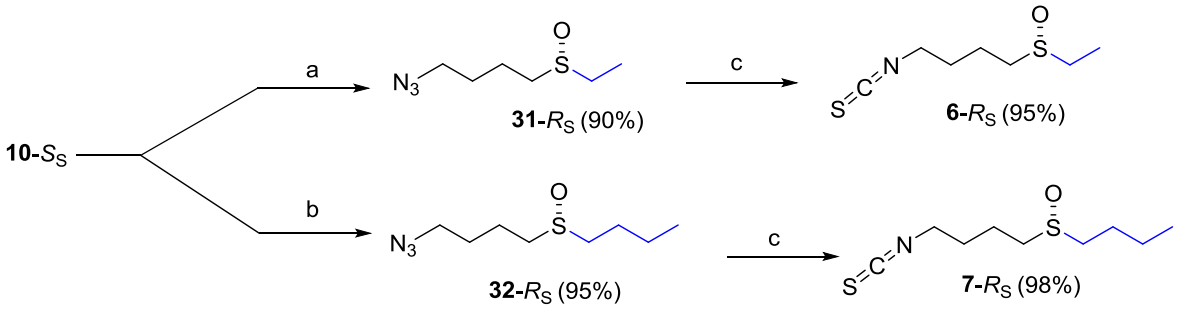
Scheme 3: Enantiodivergent synthesis of (*S*)- and (*R*)-sulfinate esters **10**, **26**, and **27** using the DAG-methodology



Scheme 4: Synthesis of enantiopure natural sulforaphane **2**-R_S and homologues **4**-R_S and **5**-R_S: a) CH_3MgBr, toluene b) (i) PPh_3, Et_2O, reflux, (ii) CS_2, reflux.



Scheme 5: Synthesis of enantiopure unnatural sulforaphane **2**-S_S and homologues **4**-S_S and **5**-S_S: a) CH_3MgBr, toluene b) (i) PPh_3, Et_2O, reflux, (ii) CS_2, reflux.



Scheme 6: Synthesis of enantiopure sulforaphane analogues **6**-R_S and **7**-R_S: a) C_2H_5MgBr, toluene b) C_4H_7MgBr, toluene, c) (i) PPh_3, Et_2O, reflux, (ii) CS_2, reflux.

Table 1. Selective cytotoxic effect of sulforaphane analogues on tumoral (A549) and normal (MRC-5) cells.

Entry	Compound	A549 IC_{50} (μM)	MRC-5 IC_{50} (μM)	*P*
1	**lactic acid**	26.61[a]	23.93[a]	0.1811
2	**Cisplatin**	11,67	115,71	
3	**2**-R_S	19.60	46.58	0.0090
4	**4**-R_S	9.62	18.54	0.0050
5	**4**-S_S	12.00	20.25	0.0100
6	**5**-R_S	10.06	16.92	0.0390
7	**5**-S_S	7.54	17.58	0.0030
8	**6**-R_S	19.78	40.79	0.0020
9	**7**-R_S	9.77	17.44	0.0001

[a]IC_{50} (mM)