Design and Synthesis of Bifunctional Isothiocyanate Analogs of Sulforaphane:† Correlation between Structure and Potency as Inducers of Anticarcinogenic Detoxication Enzymes‡

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Thirty-five bifunctional isothiocyanates were synthesized as structural analogs of sulforaphane [(−)-1-isothiocyanato-4(*R*)-(methylsulfinyl)butane] that was recently isolated from broccoli as the principal and very potent inducer of detoxication (phase 2) enzymes in mouse tissues and murine hepatoma cells (Hepa 1c1c7) in culture (Zhang, Y.; Talalay, P.; Cho, C.-G.; Posner, G. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399–2403). Determination of the potency of each analog in inducing NAD(P)H:quinone reductase, a phase 2 detoxication enzyme, has allowed generalizations concerning the relation of structure and activity. The most potent analogs were bifunctional derivatives in which the isothiocyanate group was separated from a methylsulfonyl or an acetyl group by three or four carbon atoms, and in some of which these groups were conformationally restricted. Among these analogs, the bicyclic ketoisothiocyanate (±)-*exo*-2-acetyl-6-isothiocyanatonorbornane (**30**) was a very potent inducer (comparable to sulforaphane) of quinone reductase in hepatoma cells, and it also induced both quinone reductase and glutathione transferases in several mouse organs *in vivo*. This and related bicyclic ketoisothiocyanates represent potent phase 2 enzyme inducers that are relatively easily synthesized and that may be more stable metabolically than the natural sulfoxide sulforaphane.

The fate of chemical carcinogens *in vivo* is determined at least in part by the balance between phase 1 enzymes (cytochromes P-450) that activate many carcinogens to highly reactive electrophilic metabolites capable of damaging DNA and phase 2 enzymes (e.g. glutathione transferases, NAD(P)H:quinone oxidoreductase [QR], UDP-glucuronosyltransferases) that convert these reactive electrophiles to less toxic and more easily excretable products.[1-3] A wide variety of protectors against chemical carcinogenesis are also inducers of phase 2 enzymes in many animal cells and tissues, and there is convincing evidence that monofunctional induction of phase 2 enzymes is a major mechanism responsible for such protection. It is therefore of interest that vegetables, and especially crucifers, are rich in inducer activity and contain a variety of inducer molecules. By the use of a simple screening procedure involving measurement of quinone reductase activities of murine hepatoma cells grown in microtiter plates,[4,5] we have recently isolated and identified sulforaphane [(−)-1-isothiocyanato-4(*R*)-(methylsulfinyl)-butane] as the principal and very potent phase 2 enzyme inducer from SAGA broccoli.[6] A number of natural and synthetic isothiocyanates have been shown to block the neoplastic activity of a variety of carcinogens in rodents, to induce phase 2 enzymes *in vivo* and in cells in culture, and to inhibit metabolic activation of certain carcinogens.[7] In an effort to understand the unusually high potency of sulforaphane as an enzyme inducer, we designed, synthesized, and evaluated the activities *in vitro* of a number of isothiocyanates, each carrying one additional polar group. We also determined the inducer potency in mouse tissues of (±)-*exo*-2-acetyl-6-isothiocyanatonorbornane (**30**), one of the most potent synthetic analogs of sulforaphane developed in this study.

Results

Acyclic Analogs of Sulforaphane in Which the Methylsulfinyl Group Has Been Replaced by Other Polar Groups. We have recently shown that sulforaphane, isolated from broccoli, is an extremely potent inducer of QR, equivalent in potency to synthetic racemic sulforaphane.[6] The concentration of sulforaphane required to double the QR activity (CD value) was 0.2 μM. A limited structure–activity study of the analogs $CH_3S(O)_m(CH)_nN{=}C{=}S$, where m = 0, 1, or 2 and n = 3, 4, or 5, led to the following conclusions: (a) sulforaphane is the most potent inducer; (b) the sulfoxides and sulfones do not differ much in potency, but they are more potent than the sulfides; and (c) compounds with four or five methylene groups bridging the methylsulfur and isothiocyanate functions are more potent than those containing only three methylene groups.[6]

The polar sulfoxide group of sulforaphane (CD = 0.2 μM) is clearly very important for inducer activity, since *n*-hexyl isothiocyanate in which the sulfoxide functionality is replaced by a methylene group is much less potent (CD = 15 μM; Table 1). Measurements of the inducer potencies (Table 1) of analogs of the type $Z(CH_2)_4N{=}C{=}S$, synthesized for these studies, support the following conclusions: (a) of the eight synthetic analogs, only two are very potent; (b) of these two, the methyl ketone **4** and the dimethylphosphine oxide **8** are almost equal in potency to sulforaphane; and (c) whereas the methyl ketone analog

† These bifunctional isothiocyanates are the subject of a pending U.S. patent application.

‡ Abbreviations and trivial names: QR, quinone reductase [NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2]; GST glutathione *S*-transferase, EC 2.5.1.18; CD value, the concentration of an inducer required to double the specific activity of quinone reductase in Hepa 1c1c7 murine hepatoma cells; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.

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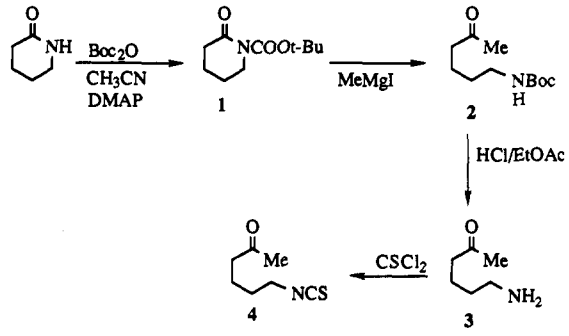
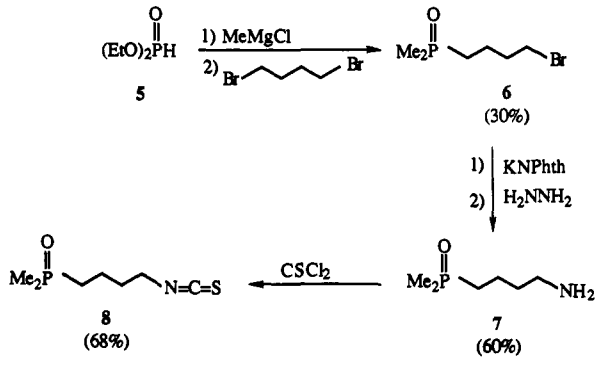
Table 1. Effect of Replacing the Methylsulfinyl Group of Sulforaphane on Inducer Potency for QR in Murine Hepatoma Cells

Z	CD	Z	CD
Et	15.0	MeOOC	2.8
$CH_3S(O)$ (sulforaphane)	0.2	MeSCO	2.8
N≡C	2.0	MeCO	0.2
HOOC	2.2	*n*-BuCO	2.0
		$Me_2P(=O)$	0.4
$CH_3S(O)CH=CH(CH_2)_2NCS$ (sulforaphene)	0.4		

Scheme 1



Scheme 2

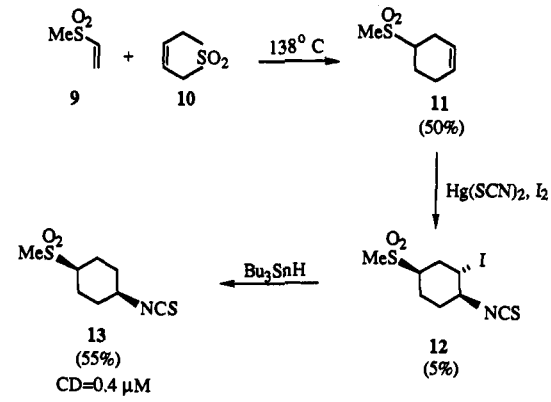


4 is a potent inducer, the corresponding *n*-butyl ketone is not a good inducer. At present, we do not understand the reason(s) for the differences in potencies among these analogs. The methods of preparation of the most potent analogs are outlined in Schemes 1 and 2.

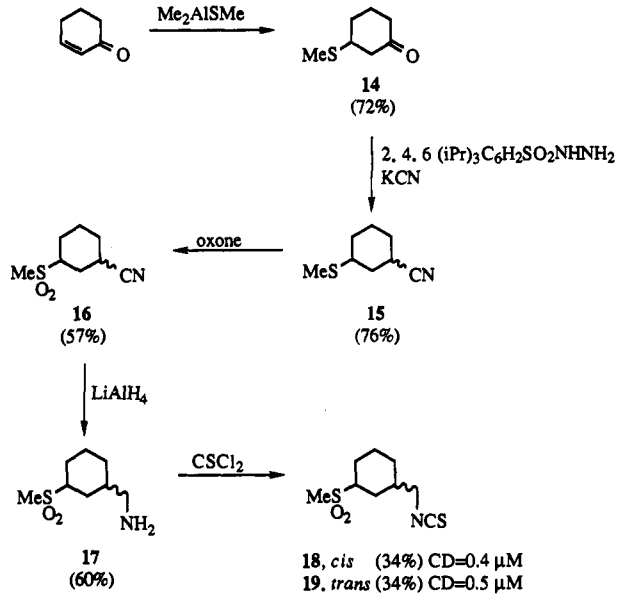
Nonaromatic Cyclic Analogs with Restricted Conformations. A. Monocyclic Analogs. Cyclohexyl isothiocyanate is a relatively weak inducer (CD = 56 μM). Using general literature procedures, we prepared three methylsulfonyl isothiocyanates (13, 18, 19) in which the two polar functionalities are separated by four carbon atoms and in which a cyclohexane ring restricts the conformational mobility of the two functional groups (Schemes 3 and 4). Sulfones were targeted because they are more stable than sulfoxides toward several of the reaction conditions used in Schemes 3 and 4 and also because the sulfone erysolin, $CH_3SO_2(CH_2)_4NCS$, is only about 2-fold less potent than the sulfoxide sulforaphane.[6] The CD values of these conformationally restricted synthetic sulfonyl isothiocyanates 13, 18, and 19 ranged from 0.4 to 0.5 μM, indicating considerable potency and emphasizing the importance of the presence of the polar sulfonyl group, although not its spatial relation to the isothiocyanate function.

B. Bicyclic Analogs. Commercial *exo*-norbornyl isothiocyanate (CD = 32 μM) is a somewhat more potent

Scheme 3



Scheme 4



inducer than cyclohexyl isothiocyanate (CD = 56 μM). The dramatic increase in inducer potency observed by the addition of a methylsulfonyl group to the six-membered monocyclic system was also observed in the bicyclic norbornane analogs. Starting with commercially available 5-acetyl-2-norbornene or easily made 5-(methylsulfonyl)-2-norbornene, we added the elements of H-SCN across the strained carbon–carbon double bond of these norbornenes[8] to produce directly as the major products the mixture of positional and orientational isomers shown in Scheme 5. Chromatographic separations provided the pure bifunctional products listed in Table 2. Assignment of position and orientation of the two functional groups in each product was based on ^1H and ^{13}C NMR spectroscopy (see the Experimental Section); single-crystal X-ray crystallography confirmed the structure of bifunctional norbornane 23.

Several aspects of the data in Table 2 are noteworthy: (1) 9 of the 12 bifunctional compounds in this table have CD values of less than 1.0, indicative of high enzyme induction potency; (2) where direct comparison is possible, the compounds with the *exo*-oriented Z-substituents are more potent than those with *endo*-oriented Z-substituents (i.e. **29** > **20**, **30** > **22**); and (3) as was found in the acyclic series (*cf.* methyl ketone 4), the methyl ketone (i.e. acetyl) functionality in bicyclic analogs **22**, **23**, and **30** contributes significantly to making these bifunctional compounds very potent inducers.

Scheme 5

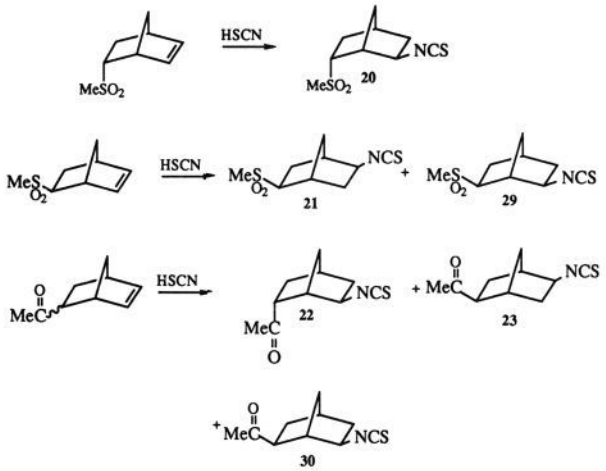


Table 2. Potency of Norbornyl Isothiocyanates in Inducing QR in Murine Hepatoma Cells

Four-Carbon Link between Functionalities

compd	*endo* Z	CD (μM)	compd	*exo* Z	CD (μM)
			21	$MeSO_2$	0.7
			23	MeCO	0.4
			24	N≡C	0.6
			25	O_2N	1.1
			26	MeOOC	0.7
			27	MeCH(OH)	0.5
28	OH	19			

Three-Carbon Link between Functionalities

compd	*endo* Z	CD (μM)	compd	*exo* Z	CD (μM)
20	$MeSO_2$	1.0	**29**	$MeSO_2$	0.2
22	MeCO	0.8	**30**	MeCO	0.3
			31	MeOOC	1.6

Induction of Quinone Reductase and Glutathione Transferase in Mouse Tissues by Keto Isothiocyanate 30. When synthetic (±)-*exo*-2-acetyl-6-isothiocyanatonorbornane (**30**) was administered to female CD-1 mice by gavage in doses of 7.5, 15, or 30 μmol daily for 5 days, the QR and GST (measured with both 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene), specific activities of the cytosols of liver, forestomach, glandular stomach, and proximal small intestine were increased in a dose-dependent manner (Figure 1). The increases in specific activities at the highest doses were generally about 2–4-fold, except for the inductions of GST in the small intestine (measured with DCNB) which were considerably higher (14–16-fold). We conclude that the keto isothiocyanate **30** induces QR not only in murine hepatoma cells, but also, like sulforaphane,[6] induces QR and GST activities in a number of murine organs. Insofar as quantitative comparisons can be made, keto isothiocyanate **30** and sulforaphane do not differ much in inducer potencies or organ-response patterns.

Aromatic Analogs. A. Aryl Isothiocyanates. As expected based on previous negative enzyme induction results with phenyl isothiocyanate,[3] none of the *ortho*-substituted phenyl isothiocyanates we have prepared (including *o*-OMe, -Cl, -CH_2CH_2SMe) has any significant inducer potency under standard assay conditions.

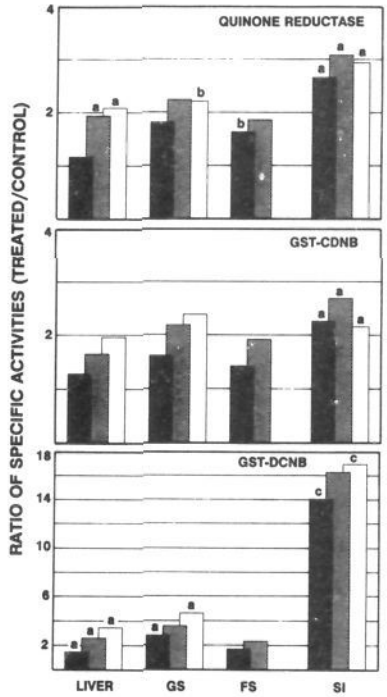


Figure 1. Effect of oral administration of *exo*-2-acetyl-6-isothiocyanatonorbornane (**30**) on the specific activities of cytosolic glutathione transferases measured with CDNB (GST-CDNB) and DCNB (GST-DCNB) and quinone reductase of liver, glandular stomach (GS), forestomach (FS), and proximal small inte stine (SI) of mice. The compound was administered to 6-week-old female CD-1 mice in 0.1 mL of Emulphor EL620P (GAF, Linden, NJ) daily for 5 days in the following quantities: 7.5 μmol (solid bar), 15 μmol (hashed bar), or 30 μmol (open bar). Four or five animals were studied in each of the treated groups and 10 in the control group. The results are expressed as the ratios (± SEM) of the specific activities of organ cytosols from treated to controls receiving the vehicle only. Cytosols were prepared from the tissues 24 h after the last treatment and assayed for enzyme activities. The enzyme specific activities (nmol·min^{-1}·mg^{-1} ± SEM) of the cytosols of control mice were as follows. Liver: GST-CDNB, 1080 ± 51.9; GST-DCNB, 10.9 ± 0.78; QR, 57.3 ± 4.1. Glandular stomach: GST-CDNB, 908 ± 27.5; GST-DCNB, 4.5 ± 0.18; QR, 2630 ± 190. Forestomach: GST-CDNB, 1780 ± 57.2; GST-DCNB, 7.6 ± 0.60; QR, 1040 ± 62.9. Small intestine: GST-CDNB, 685 ± 57.9; GST-DCNB, 0.89 ± 0.23; QR, 488 ± 45.9. The relative standard errors of the induction ratios [(SEM/mean) × 100] are as follows: no designation, ± 0–10%; *a*, ± 10–20%; *b*, ± 20–30%. The unknown reasons, the forestomachs of mice receiving 30 μmol of **30** were significantly heavier (89.4 ± 3.9 mg, wet weight) than the controls (42.4 ± 2.5 mg) or the forestomachs of mice receiving either 7.5 or 15 μmol of 30 (mean of 46.9 mg). For cytosols of the forestomach homogenates of animals receiving 30 μmol of **30** also contained more protein than the other groups. The specific activities of QR and GST in the cytosols of forestomachs of mice receiving the 30-μmol dose were considerably lower than those treated with the lower does of 30 and approached control values. The results obtained with the 30-μmol dose are omitted from the figure.

B. Benzylic Isothiocyanates. Prompted by a report by Kjaer on *o*-methoxybenzyl isothiocyanate,[9] we prepared a series of benzylic isothiocyanates substituted in the *ortho* position by the following substituents (CD values [μM] in parentheses): CH_2SCH_3 (4.3), OMe (2.4), NMe_2 (12.5), SMe (6.8), F (13.1), Cl (14.1), Br (12.5), Me (3.7), Et (12.5),

OEt (2.5). None of these analogs was a particularly active inducer or even better than benzyl isothiocyanate itself (CD = 2–3 μM).

Conclusion. In conclusion, several easily prepared, conformationally restricted, non-sulfoxide, nonaromatic, bifunctional analogs of sulforaphane have been identified and have been shown *in vitro* to possess high phase 2 enzyme inducer potency. One of the most promising lead compounds, bicyclic keto isothiocyanate **30**, was shown also *in vivo* to induce QR and GST activities in a number of murine organs. Such keto isothiocyanates, probably more stable toward biological oxidation and reduction than sulfoxide–isothiocyanates like sulforaphane, may be useful for protection against cancer.

Experimental Section

General Methods. Thiophosgene was purchased from Carbolabs Inc. (Bethany, CT), and all other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and, unless otherwise specified, were used as received without further purification. Benzyl isothiocyanate, obtained from the Aldrich Chemical Co., was distilled under reduced pressure prior to use. Other isothiocyanates which were not synthesized for this project were obtained from Trans World Chemicals (Rockville, MD). ^1H and ^{13}C NMR spectra were determined on a Bruker AMX 300 MHz or a Varian XL-400 MHz spectrometer. High-resolution mass spectra were obtained at 70 eV on a VG-70S mass spectrometer. FT-IR spectra were recorded on a Perkin-Elmer Model 1600 FT-IR spectrophotometer. Elemental analyses were performed by Atlantic Microlab, Norcross, GA. Medium-pressure column chromatography was performed with silica gel (EM SCIENCE, 230–400 mesh). High-pressure liquid chromatography was performed with RAININ HPXL chromatography using a semi-prep silica column.

Boc-Protected Lactam 1. To a flask charged with 0.56 g (5.7 mmol) of δ-valerolactam, 60 mg (0.5 mmol) of 4-(dimethylamino)-pyridine (DMAP), and 30 mL of acetonitrile was added 1.22 g (5.7 mol) of di-*tert*-butyl dicarbonate (Boc_2O) at room temperature (RT). After 4 h at RT, the reaction mixture was concentrated and partitioned between ether and 1 M $KHSO_4$. The separated organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to give 0.90 g of product as a bright yellowish green liquid (used for the next reaction without purification).

4-Boc-aminobutyl Methyl Ketone (2). To protected lactam 1 in 10 mL of THF was added 2 mL of MeMgI (3 M in ether, 6 mmol) slowly at −78 °C. After 3 h at −78 °C, the reaction mixture was quenched with aqueous NH_4Cl, and the resulting solution was extracted with ether (2 × 20 mL). The combined ether solution was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give crude methyl ketone 2 as a brown oil (used for the next reaction without further purification).

4-Aminobutyl Methyl Ketone (3). To methyl ketone 2 dissolved in 3 mL of EtOAc was added 1 mL of 37% of HCl at RT. After 30 min, the reaction mixture was diluted with 5 mL of H_2O and washed with ether. The aqueous solution was then strongly basified with solid NaOH and extracted with $CHCl_3$ (2 × 20 mL). The organic solution was dried over K_2CO_3 and concentrated to give a smelly light brown oil (used for the next reaction without purification).

2-Oxohexyl Isothiocyanate (4). To amino methyl ketone 3 in 2 mL of H_2O and 2 mL of $CHCl_3$ were added 0.16 mL of $CSCl_2$ (1.58 mmol) and 2 mL of 5% NaOH at RT. After 30 min the reaction mixture was diluted with 10 mL of $CHCl_3$, and the decanted organic layer was dried over $MgSO_4$, concentrated, and chromatographed (8/2 Hex/EtOAc) to give 42 mg of isothiocyanate 4 as a brown oil in an overall yield of 6% from lactam 1: ^1H NMR (400 MHz, $CDCl_3$) δ 3.54–3.49 (m, 2H), 2.53–2.46 (m, 2H), 2.16 (s, 3H), 1.72–1.68 (m, 4H); FT-IR ($CHCl_3$) 3019, 2191, 2112, 1715, 1224 cm^{-1}; ^{13}C NMR (400 MHz, CD Cl_3) δ 207.8, 44.8, 42.4, 29.9, 29.3, 20.6; HRMS calcd for $C_7H_{11}NOS$ 157.0561, found 157.0565.

(4-Bromobutyl)dimethylphosphine Oxide (6). To a 25-mL flame-dried round-bottomed flask charged with 15.2 mL (45.6 mmol) of MeMgCl (3.0 M in THF) was added 1.5 mL (11.41 mmol) of diethyl phosphite (5) while the internal temperature was maintained around 25 °C with occasional cooling with an ice–water bath. After 1 h, the mixture was cannulated into a separate flask charged with 2.55 mL (22.82 mmol) of 1,4-dibromobutane and 15 mL of THF at 0 °C under an Ar atmosphere. Upon addition, the reaction mixture was heated under reflux for 5 h, cooled, and dumped into 30 mL of cold dilute HCl. The resulting aqueous solution was extracted with $CHCl_3$ (3 × 50 mL), and the combined organic solution was washed with saturated K_2CO_3, dried over K_2CO_3, and concentrated in vacuo to give 2.48 g of crude product as a tan oil. Purification by flash column chromatography (silica gel, 8/2 EtOAc/MeOH → 6/4 EtOAc/hexane) afforded 0.72 g of phosphine oxide 6 as a colorless oil (used for the next reaction without purification).

(4-Aminobutyl)dimethylphosphine Oxide (7). In a 100-mL round-bottomed flask were placed 0.733 g (3.44 mmol) of phosphine oxide 6, 0.766 g of potassium phthalimide, and 20 mL of DMF. The mixture was heated under reflux for 4 h, cooled, and dumped into 60 mL of $CHCl_3$. The organic solution was washed with H_2O, dried over $NaHCO_3$, and concentrated in vacuo to afford 0.92 g of product phthalimide as a white solid. To a separate flask charged with 0.10 g of product phthalimide was added 4 mL of methanolic hydrazine (0.2 M in MeOH) at RT. After 14 h at RT, the reaction mixture was concentrated, and the residue was treated with 5 mL of 1 N HCl, washed with $CHCl_3$, and strongly basified with solid NaOH. The basified solution was then extracted with $CHCl_3$ (2 × 20 mL), and the combined organic solution was dried over K_2CO_3 and concentrated in vacuo to give 33 mg of amine phosphine oxide 7 as a white solid (used for the next reaction without further purification).

(4-Isothiocyanatobutyl)dimethylphosphine Oxide (8). To a flask charged with 33 mg (0.22 mmol) of amine 7 and 1 mL of $CHCl_3$ were added at RT 0.02 mL (0.27 mmol) of $CSCl_2$ and 0.3 mL of 1 N NaOH. After 35 min at RT, the reaction mixture was partitioned between 10 mL of $CHCl_3$ and 10 mL of H_2O. The separated organic layer was dried over $MgSO_4$, concentrated in vacuo, and chromatographed (8/2 EtOAc/MeOH) to afford 29 mg of isothiocyanate 8 as a reddish yellow oil in 68% yield: ^1H NMR (400 MHz, $CDCl_3$) δ 3.54 (t, J = 6.0 Hz, 2H), 1.82–1.70 (m, 6H), 1.48 (s, 3H), 1.44 (s, 3H); FT-IR ($CHCl_3$) 2941, 2191, 2097, 1302, 1173 cm^{-1}; ^{13}C NMR (400 MHz, $CDCl_3$) δ 44.5, 30.6 (d, J = 20.2 Hz, 1C), 30.7 (d, J = 34.7 Hz, 1C), 19.3, 16.2 (d, J = 69 Hz, 2C); ^{31}P NMR ($CDCl_3$) δ 46.1; HRMS calcd for $C_7H_{14}NOPS$ 191.0534, found 195.0536.

4-(Methylsulfonyl)cyclohexene (11). In a sealed tube were placed 0.50 g (5.5 mmol) of methyl vinyl sulfone (9), 0.67 g (5.5 mmol) of 1,4-but-2-enediyl sulfone (10) and 2 mL of absolue EtOH. After 2 days at 138 °C, the reaction mixture was cooled and poured into aqueous Na_2CO_3. After 10 min with vigorous stirring, the aqueous solution was extracted with ether (2 × 10 mL), dried over $MgSO_4$, concentrated in vacuo, and chromatographed (1/1 hexane/ether) to give 0.20 g (2.2 mmol, 40% recovery) of methyl vinyl sulfone (9) and 0.40 g of sulfone 11 as a brown oil in 50% yield.

Iodo Isothiocyanate 12. To a flask charged with 342 mg (0.63 mmol) of $Hg(SCN)_2$ was added a premixed solution of I_2 in 8 mL of benzene. After 30 min at RT, to this mixture was added 202 mg (1.26 mmol) of cyclohexene 11 dissolved in 1 mL of benzene, and the flask containing the reaction mixture was wrapped with aluminum foil and stirred for 7.5 days at RT under an argon atmosphere. The reaction mixture was then filtered off, and the solid material was washed with ether. The ether solution was washed with aqueous KI, aqueous $Na_2S_2O_3$, and brine successively, dried over $MgSO_4$, and concentrated in vacuo. Flash column chromatography (1/1 ether/hexane) afforded 20 mg of iodide 12 (5% yield) as a brown oil along with three other isomers (11% yield).

Sulfonyl Isothiocyanate 13. To a flask charged with 21 mg (0.07 mmol) of iodide 12 and 1 mL of benzene was added 0.05 mL (0.2 mmol, 3 equiv) of Bu_3SnH at RT. After 10 h, the reaction mixture was treated with 2 mL of wet ether and 35 mg (0.2 mmol) of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene). The resulting mixture was filtered off, concentrated in vacuo and chromato-

graphed (100% ether → 1/1 ether/EtOAc) to afford 7.3 mg of isothiocyanate 13 as white solid (mp 123 °C) in 55% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.11–4.08 (m, 1H), 2.85 (s, 3H), 2.87–2.80 (m, 1H, overlapped), 2.23–2.18 (m, 4H), 1.93 (dd, J = 14.8, 4.4 Hz, 1H), 1.87 (dd, J = 13.2, 3.2 Hz, 1H), 1.67 (tt, J = 13.2, 3.6 Hz, 2H); FT-IR ($CHCl_3$) 3025, 2943, 2261 , 2085, 1302 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 133.5, 61.2, 52.3, 36.8, 30.5, 20.6; HRMS calcd for $C_8H_{13}NO_2S_2$ 219.0388, found 219.0391.

3-Cyanocyclohexyl Methyl Sulfide (15). Into a 100-mL round-bottomed flask were placed 0.438 g (3.0 mmol) of 14,[11] 1.418 g (4.8 mmol) of 2,4,6-triisopropylbenzenesulfonyl hydrazide,[10] and 8 mL of MeOH at RT. After 1 h, 0.739 g (11.3 mmol) of KCN was added at RT, and the resulting mixture was heated under gentle reflux for 3 h. The reaction mixture was cooled, diluted with 20 mL of H_2O, and extracted with CH_2Cl_2 (2 × 20 mL). The organic solution was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, concentrated in vacuo, and purified by flash column chromatography (8/2 hexane/EtOAc) to afford 0.360 g of sulfide 15 as a yellow oil in 76% yield.

3-Cyanocyclohexyl Methyl Sulfone (16). To a flask charged with 0.36 g (2.32 mmol) of sulfide 15 and 10 mL of aqueous MeOH (9/1 v/v MeOH/H_2O) was added 2.75 g (4.64 mmol) of Oxone (Aldrich, $2KHSO_5$·$KHSO_4$·K_2SO_4) at RT. After 24 h, the reaction mixture was filtered through a sintered-glass funnel, and the filtered solid material was washed with 50 mL of $CHCl_3$. The combined organic solution was washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo to afford 0.246 g of sulfone 16 (57% yield) as a colorless oil. This material was used in the next reaction without purification.

3-(Aminomethyl)cyclohexyl Methyl Sulfone (17). To a suspension of 0.098 g (2.59 mmol) of $LiAlH_4$ in 10 mL of anhydrous ether was cannulated 0.246 g (1.31 mmol) of nitrile 16 dissolved in 3 mL of THF at RT. Upon addition, the reaction mixture was heated under reflux. After 2.5 h, the reaction mixture was cooled, quenched with 0.5 mL of H_2O and 0.5 mL of 5% NaOH, and filtered through a sintered-glass funnel. The solid material filtered was thoroughly washed with ether. The combined organic solution was dried over K_2CO_3 and concentrated in vacuo to afford 0.150 g of amine 17 (60% yield) as a colorless oil. This material was used in the next reaction without purification.

***cis*-(3-(Methylsulfonyl)cyclohexyl)methyl Isothiocyanate 18 and *trans*-(3-(Methylsulfonyl)cyclohexyl)methyl Isothiocyanate 19.** To a flask charged with 0.15 g (0.78 mmol) of amine 17 and 3 mL of $CHCl_3$ were added 0.07 mL (0.92 mmol) of $CSCl_2$ and 1.5 mL of 5% NaOH at RT. After 1 h, the reaction mixture was diluted with 10 mL of CH_2Cl_2, washed with H_2O and brine, dried over $MgSO_4$, concentrated in vacuo, and chromatographed (1/1 hexane/EtOAc) to give 0.123 g of products (67% yield) as a mixture of isothiocyanates 18 and 19 (1:1 ratio). HPLC (40/60 EtOAc/hexane) separation afforded analytically pure 18 and 19 (both as a colorless oil). 18: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.47 (d, J = 6.0 Hz, 2H), 2.92–2.82 (m, 1H), 2.84 (s, 3H), 2.28–2.20 (m, 2H), 2.04 (tt, J = 6.8, 3.0 Hz, 1H), 1.87–1.75 (m, 2H), 1.53–1.27 (m, 3H), 1.06 (tq, J = 12.2, 3.6 Hz, 1H); FT-IR ($CHCl_3$) 3025, 2931, 2861, 2191, 2097, 1449, 1308 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 56.5, 45.5, 32.6, 32.5, 23.9, 23.8, 20.0, 19.1; HRMS calcd for $C_9H_{15}NO_2S_2$ 233.0544, found 233.0548. 19: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.50 (d, J = 6.8 Hz, 2H), 3.09–3.03 (m, 1H), 2.88 (s, 3H), 2.45–2.37 (m, 1H), 2.14–2.07 (m, 1H), 1.98–1.84 (m, 4H), 1.74–1.66 (m, 1H), 1.59–1.41 (m, 2H); FT-IR ($CHCl_3$) 3013, 2943, 2872, 2191, 2097, 1449, 1308 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 52.6, 43.5, 33.6, 28.0, 22.5, 22.1, 19.5, 14.9; HRMS calcd for $C_9H_{15}NO_2S_2$ 233.0544, found 233.0545.

***2-endo*-Methylsulfonyl Isothiocyanate 20.** In a 20-mL hydrolysis tube were placed 349 mg (2.03 mmol) of 5-*endo*-(methylsulfonyl)-2-norbornene,[12] 394 mg (4.05 mmol) of KSCN, 319 mg (3.25 mmol) of H_2SO_4, and 0.1 mL of H_2O at RT via literature precedent.[13] The tube was sealed and shaken vigorously for 5 min to give a finely divided yellow suspension. After 4 days at 50 °C, the reaction mixture was filtered through a sintered-glass funnel, and the brown solid was washed with ether. The combined organic solution was washed with H_2O, brine, dried over $MgSO_4$, and concentrated to give 270 mg of crude product containing unreacted starting material as the major component. Flash column chromatography (1/1 hexane/EtOAc) afforded 47 mg of isothiocyanate 20 as a white solid. Recrystallization from a 2:1 mixture of hexane and ether gave 23 mg of isothiocyanate 20 (5% yield) as white needles (mp 110–111 °C). The *endo* orientation of the methylsulfonyl group was assigned based on its ^{1}H NMR chemical shift (δ 2.84) being upfield from that of the corresponding *exo* methylsulfonyl groups of 21 and 28 (δ 2.86–2.87).[14] The 1,3-relationship of the two substituents was assigned based on the difference in chemical shifts between the bridgehead hydrogens (Δδ = 0.2) vs that in the 1,4-positional isomer 21 (Δδ = 0.1):[15] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.79 (dd, J = 7.6, 4.4 Hz, 1H), 3.30–3.25 (m, 1H), 2.90–2.84 (m, 1H), 2.84 (s, 3H), 2.68 (d, J = 4.4 Hz, 1H), 2.02 (ddd, J = 13.1, 10.1, 4.2 Hz, 1H), 1.90–1.86 (m, 1H), 1.72–1.52 (m, 4H); FT-IR ($CHCl_3$) 3 025, 2120, 2097, 1320 cm^{-1}. Anal. ($C_8H_{13}NO_2S_2$) C, H, N, S.

***2-exo*-Methylsulfonyl Isothiocyanate 21 and *2-exo*-Methylsulfonyl Isothiocyanate 29.** The same procedure as described for 20 was used except that the reaction mixture was stirred for 6 days at 65 °C. After workup, isothiocyanates 21 (17% yield) and 29 (5% yield) were isolated by flash column chromatography (100% ether → 100% EtOAc). Isothiocyanate 21 was recrystallized from CH_2Cl_2/ether/hexane to afford ivy leaf-shaped crystals (mp 142–143 °C) in 12% yield. Isothiocyanate 29 was recrystallized from ether to afford small needles (mp 82–82.5 °C) in 4% yield. The assignment of positional isomers was done as described for isothiocyanate 20. Isothiocyanate 21: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (t, J = 6.8 Hz, 1H), 2.90 (bs, 1H), 2.86 (s, 3H), 2.80 (dd, J = 8.0, 5.2 Hz, 1H), 2.66 (bd, J = 5.2 Hz, 1H), 2.12 (td, J = 14.0, 5.2 Hz, 1H), 2.03 (dt, J = 12.0, 2.2 Hz, 1H), 1.88–1.84 (m, 2H), 1.68–1.60 (m, 2H); FT-IR ($CHCl_3$) 3025, 2120, 2073, 1320 cm^{-1}. Anal. ($C_8H_{13}NO_2S_2$) C, H, N, S. Isothiocyanate 29. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (dd, J = 6.8, 2.8 Hz, 1H), 2.98 (bs, 1H), 2.87 (s, 3H), 2.76 (dd, J = 6.8, 1.2 Hz, 1H), 2.58 (bs, 1H), 2.06–1.61 (m, 6H); FT-IR ($CHCl_3$) 3025, 2978, 2191, 2120, 2085, 1349, 1308, 1138 cm^{-1}; ^{13}C NMR (400 MHz, $CDCl_3$) δ 132.2, 61.5, 58.0, 45.5, 39.6, 39.2, 35.3, 33.7, 31.2; HRMS calcd for $C_8H_{13}NO_2S_2$ 231.0388, found 231.0390.

***2-endo*-Acetyl Isothiocyanate 22, *2-exo*-Acetyl Isothiocyanate 23, and *2-exo*-Acetyl Isothiocyanate 30.** To a 100-mL three-neck round-bottomed flask equipped with a magnetic stirring bar, dropping funnel, and reflux condenser were placed 2.0 g (14.7 mmol) of 5-acetyl-2-norbornene (mixture of *endo* and *exo*, Aldrich Chemical Co.), 2.86 g (29.4 mmol) of KSCN, and 10 mL of benzene. To this solution was added at RT a mixture of 2.1 g (21.5 mmol) of concentrated sulfuric acid and 1.0 mL of water slowly using a dropping funnel. After 4 days at 50 °C, the reaction mixture was filtered through a sintered-glass funnel. The filtered white solid was washed with 50 mL of ether. The combined organic solution was then washed with water and brine successively, dried over $MgSO_4$, and concentrated in vacuo to afford a tan oil. Subsequent purification via flash column chromatography (2/8 ether/hexane) afforded 1.73 g of product (60% yield, colorless oil) as a mixture of four stereoisomers. Purification by HPLC (97/3 hexane/EtOAc, 10 mL/min) gave isothiocyanates 22 (10% yield), 23 (23% yield), and 30 (22% yield). The assignment of positional and orientational isomers was done as described for isothiocyanates 20, 21, and 29. The structure of isothiocyanate 23 was confirmed by X-ray crystallography. Isothiocyanate 22: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.51 (dd, J = 7.6, 2.8 Hz, 1H), 2.85–2.79 (m, 1H), 2.68–2.54 (m, 1H), 2.43 (d, J = 4.8 Hz, 1H), 2.06 (s, 3 H), 1.74–1.70 (m, 1H), 1.66 (ddd, J = 13.6, 7.6, 2.4 Hz, 1H), 1.59–1.43 (m, 4H); FT-IR ($CHCl_3$) 3013, 2955, 2132, 2097, 1702, 1343 cm^{-1}. Anal. ($C_{10}H_{13}NOS$) C, H, N, S. Isothiocyanate 23: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 (dd, J = 7.2, 2.8 Hz, 1H), 2.57 (d, J = 4.4 Hz, 1H), 2.51 (d, J = 4.8 Hz, 1H), 2.34 (dd, J = 8.8, J = 5.2 Hz, 1H), 2.15 (s, 3H), 1.98 (dt, J = 13.2, 4.8 Hz, 1H), 1.88 (ddd, J = 13.2, 7.6, 2.4 Hz, 1H), 1.75 (dt, J = 13.6, 4.4 Hz, 1H), 1.52 (ddt, J = 10.8, 4.0, 1.6 Hz, 1H), 1.33 (ddt, J = 10.8, 4.0, 1.6 Hz, 1H), 1.22 (ddd, J = 13.2, 8.8, 2.0 Hz, 1H); FT-IR ($CHCl_3$) 2978, 2 179, 2146, 2085, 1708, 1449, 1343 cm^{-1}. Anal. ($C_{10}H_{13}NOS$) C, H, N, S. Isothiocyanate 30: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (dd, J = 7.6, 2.8 Hz, 1H), 2.71 (bs, 1H), 2.43 (dd, J = 4.5, 3.6 Hz, 1H), 2.31 (dd, J = 8.4, 6.0 Hz, 1H), 2.17 (s, 3H), 1.83–1.67 (m, 2H), 1.58–1.54 (m, 2H), 1.38–1.30 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 207.4, 130.1, 58.3, 50.7, 46.5, 39.9, 35.4, 33.6, 31.5, 28.7; FT-IR ($CHCl_3$) 2955, 2132, 2085, 1708, 1343 cm^{-1}; HRMS calcd for $C_{10}H_{13}NOS$ 195.0719, found 195.0719.

2-*exo*-Cyanonorbornyl Isothiocyanate 24. To a sealed tube charged with 145 mg (1.2 mmol) of 5-*exo*-cyano-2-norbornene, 238 mg (2.4 mmol) of KSCN, and 3 mL of benzene were added 177 mg (2.4 mmol) of H_2SO_4 and 0.08 mL of H_2O. After 48 h at 63 °C, the reaction mixture was diluted with ether, filtered, concentrated, and chromatographed (3/7 ether/hexane) to give 77 mg (53%) of starting material and 33 mg of product as a mixture of two isomers. Subsequent HPLC separation (9/1 hexane/EtOAc) afforded 30 mg of isothiocyanate **24** as a white solid (mp 49.5–50.5 °C from hexane) in 14% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.59 (t, J = 5.6 Hz, 1H), 2.73 (bs, 1H), 2.64 (d, J = 4.4 Hz, 1H), 2.32 (ddd, J = 9.2, 4.8, 1.6 Hz, 1H), 1.90 (dt, J = 13.6 Hz, 4.8, 1H), 1.85–1.73 (m, 5H); FT-IR ($CHCl_3$) 3021, 2979, 2954, 2240, 2201, 2146, 2100, 1452, 1349 cm^{-1}; ^{13}C NMR (400 MHz, $CDCl_3$) δ 131.0, 122.2, 57.3, 43.4, 40.8, 39.0, 34.4, 31.8, 29.9; HRMS calcd for $C_9H_{10}N_2S$ 178.0563, found 178.0561.

2-*exo*-Nitronorbornyl Isothiocyanate 25. To a flask charged with 183 mg (1.3 mmol) of 5-*exo*-nitro-2-norbornene,[16] 255 mg (2.6 mmol) of KSCN, and 6 mL of benzene were added 190 mg (2.6 mmol) of H_2SO_4 and 0.1 mL of H_2O. After 75 h at 40 °C, the reaction mixture was diluted with ether, filtered off, concentrated, and chromatographed (9/1 hexane/ether) to afford 107 mg of starting material (59%) and 36 mg of isothiocyanate **25** as a yellow solid (14%). Subsequent recrystallization from ether/hexane gave 26 mg of isothiocyanate **25** as colorless needles (mp 67–68 °C) in 10% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.32 (q, J = 8.0 Hz, 1H), 3.62 (t, J = 5.2 Hz, 1H), 3.00 (bs, 1H), 2.67 (d, J = 4.8 Hz, 1H), 2.39 (dt, J = 14.4, 4.0 Hz, 1H), 1.88–1.71 (m, 5H); FT-IR ($CHCl_3$) 3013, 2132, 2085, 1549, 1367, 1220 cm^{-1}. Anal. ($C_8H_{10}N_2O_2S$), C, H, N, S.

2-*exo*-(Methoxycarbonyl)norbornyl Isothiocyanate 26 and 2-*exo*-(Methoxycarbonyl)norbornyl Isothiocyanate 31. To a flask charged with 40 mg (0.26 mmol) of 5-*exo*-(methoxycarbonyl)-2-norbornene,[17] 51 mg (0.53 mmol) of KSCN, and 0.5 mL of benzene were added 38 mg (0.53 mmol) of H_2SO_4 and 0.02 mL of H_2O. After 46 h at 63 °C, the reaction mixture was directly chromatographed (7/3 hexane/ether) to give 20 mg (0.09 mmol) of product as a mixture of two isomers based on ^{1}H NMR analysis (**26**:**31** 85:15). Purification by HPLC (95/5 hexane/EtOAc) afforded pure isothiocyanates **26** as a white solid (ca. 31% yield, mp 39.5–40.5 °C) and **31** as a liquid (ca. 6% yield). Isothiocyanate **26**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.59 (dd, J = 7.6, 3.2 Hz, 1H), 2.63 (d, J = 3.5 Hz, 1H), 2.55 (d, J = 4.3 Hz, 1H), 2.25 (dd, J = 8.5, 4.3 Hz, 1H), 1.97 (dt, J = 13.7, 4.7 Hz, 1H), 1.85 (ddd, J = 13.4, 7.8, 1.9 Hz, 1H), 1.74 (dt, J = 13.5, 3.8 Hz, 1H), 1.62–1.53 (m, 2H), 1.42–1.36 (m, 1H); FT-IR ($CHCl_3$) 2976, 2953, 2140, 2088, 1729, 1437, 1346 cm^{-1}; ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.2, 58.0, 51 .9, 45.0, 43.8, 40.2, 40.1, 33.6, 29.8; HRMS calcd for $C_{10}H_{13}NO_2S$ 211.0667, found 211.0669. **31**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 3H), 3.62 (dd, J = 7.6, 3.6 Hz, 1H), 2.76 (bs, 1H), 2.44 (bs, 1H), 2.22 (dd, J = 8.4, 5.6 Hz, 1H), 1.88–1.40 (m, 6H); FT-IR ($CHCl_3$) 3027, 3009, 2974, 2954, 2197, 2132, 1732, 1437, 1346 cm^{-1}; ^{13}C NMR (400 MHz, $CDCl_3$) δ 174.7, 58.2, 52.1, 47.8, 42.8, 39.8, 35.4, 34.0, 33.0; HRMS calcd for $C_{10}H_{13}NO_2S$ 211.0667 found 211.0667.

***exo*-2-(1′-Hydroxyethyl)norbornyl Isothiocyanate 27.** To 37.3 mg (0.2 mmol) of acetyl isothiocyanate **23** in 1.5 mL of MeOH was added 8.7 mg (0.2 mmol) of $NaBH_4$ slowly at 0 °C. After 15 min at 0 °C, the reaction mixture was treated with a few drops of water, diluted with ether, dried over $MgSO_4$, concentrated in vacuo, and purified by prep TLC (8/2 ether/hexane) to give 21.0 mg of hydroxy isothiocyanate **27** as a mixture of diastereomers (white solid; mp 64–69 °C recrystallized from hexane) in 56% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (d, J = 6.0 Hz, CH_3), 1.10 (d, J = 6.4 Hz, CH_3); FT-IR ($CHCl_3$) 3623 , 3460, 2966, 2872, 2097, 1343 cm^{-1}. Anal. ($C_{10}H_{15}NOS$), C, H, N, S.

2-*endo*-Hydroxynorbornyl Isothiocyanate 28. To a flask charged with 0.515 g (4.54 mmol) of 5-hydroxy-2-norbornene (mixture of *endo* and *exo*, Aldrich Chemical Co.) 0.530 g (5.45 mmol) of KSCN, and 8 mL of benzene was added a premixed solution of H_2SO_4 (0.400 g) in 0.2 mL of H_2O at RT. After 3 days at 55 °C, the reaction mixture was filtered and chromatographed (40/60 ether/hexane) to give 0.075 g of product as a white solid in 10% yield. A portion of the material was recrystallized from hexane/ether for analysis (white flakes; mp 64–66 °C): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.24–4.18 (m, 1H), 3.67–3.65 (m, 1H), 2.52 (ddd, J = 13.2, 7.6, 2.0 Hz, 1H), 2.41 (d, J = 5.2 Hz, 1H), 2.35 (td, J = 5.2, 1.6 Hz, 1H), 2.00 (ddd, J = 15.6, 10.4, 5.6 Hz, 1H), 1.69 (dq, J = 14.0, 3.2 Hz, 1H), 1.57–1.53 (m, 2H), 1.45–1.42 (m, 1H), 0.76 (dt, J = 14.0, 3.2 Hz, 1H); FT-IR ($CHCl_3$) 3613 , 3472, 2966, 2097, 1343 cm^{-1}. Anal. ($C_8H_{11}NOS$) C, H, N, S.

Bioassay Procedures. Measurement of Inducer Potency in Hepta 1c1c7 Murine Hepatoma Cells. These determinations were carried out on cells grown in 96-well microtiter plates according to minor modifications[18] of the procedure of Prochaska and Santamaria.[4] The cells (10 000 per well) were grown for 24 h in medium containing 10% heat- and charcoal-treated fetal calf serum and then exposed to serial dilutions of the inducers for 48 h before measurement of QR specific activity. Compounds were dissolved in acetonitrile and diluted so that the final concentration of solvent was 0.1% by volume in all wells.

Induction of Quinone Reductase and Glutathione Transferase Activities in Mouse Tissues. Treatment of Animals. Five-week-old female CD-1 mice (Charles River Laboratories, Wilmington, MA) were acclimatized for 1 week on AIN 76A pellets diet. The animals were housed in plastic cages (four or five per cage). Each mouse received 0.1 mL of Emulphor EL620P (GAF, Linden, NJ) alone (10 mice) or 0.1 mL of Emulphor containing 7.5 μmol (4 mice), 15 μmol (5 mice), or 30 μmol (5 mice) of *exo*-2-acetyl-6-isothiocyanatonorbornane, daily by gavage for 5 days. Twenty-four hours after the last treatment, the animals were killed by carbon dioxide inhalation, the organs were removed, frozen in liquid nitrogen, and stored at −80 °C until analysis.

Preparation of Tissue Cytosols and Assay of Their Enzymatic Activities. The cytosols were prepared as described.[18,19] In the present experiments the entire proximal small intestine (after removal of contents) was homogenized rather than the mucosal scrapings.

Specific enzyme activities were measured at 25 °C as described[18,20] except that the assay systems were miniaturized (to one-tenth volume) so that measurements could be made in 96-well microtiter plates with the use of a microtiter plate reader (UVmax, Molecular Devices, Palo Alto, CA). The QR specific activities were determined in a final volume of 0.3 mL by measuring the NADPH-dependent rate of menadiol-mediated reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 610 nm. The specific activities of GST were measured with DCNB and CDNB in final volumes of 0.2 mL at 340 nm. Suitable rates were obtained by use of appropriate volumes and dilutions of cytosols and were derived from absorbance changes during the initial 2 min, based on the average of four wells. Rates were corrected for absorbance changes in wells containing all components except cytosol. Protein determinations were made according to Bradford.[21] The ratios of the mean enzyme specific activities (nanomoles of product formed per minute per milligram of protein) of tissue cytosols from animals treated with the inducer to those receiving vehicle only were then calculated (± SEM). The standard errors of these ratios were calculated.[18]

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