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Identification, Synthesis, and Enzymology of Non-natural Glucosinolate Chemopreventive Candidates

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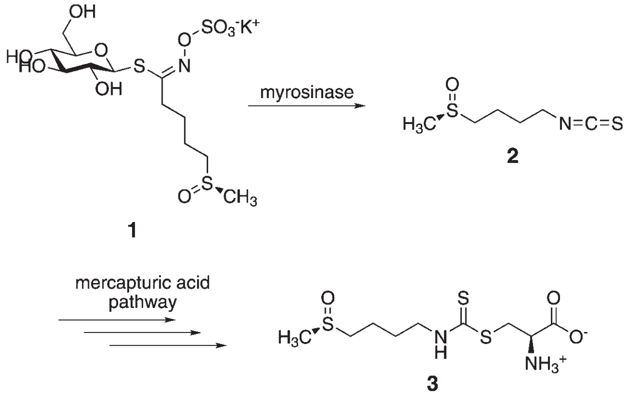
Isothiocyanates (ITCs) are one of the many classes of breakdown products of glucosinolates found in crucifers such as broccoli and are thought to be partially responsible for the reduced risk of degenerative diseases associated with the consumption of vegetables. The production of ITCs such as L-sulforaphane is dependent on the hydrolytic bioactivities of myrosinase, localized both within vegetable tissues and within flora of the human GI tract, and is associated with important cancer chemopreventive activities. We hypothesized that novel isothiocyanates with enhanced chemopreventive properties relative to L-sulforaphane could be identified and that their glucosinolate precursors could be synthesized. From a library of 30 synthetic ITCs, we identified several with bioactivities equal or superior to those of L-sulforaphane. The corresponding non-natural glucosinolate precursors to these novel ITCs were constructed and found to be substrates for myrosinase. By utilizing a novel RP-HPLC assay to monitor myrosinase-dependent hydrolysis reactions, 2,2-diphenylethyl glucosinolate and (biphenyl-2-yl)methyl glucosinolate were shown to exhibit 26.5 and 2.8%, respectively, of the relative activity of sinigrin and produced their corresponding ITCs in varying yields. These data support the notion that non-natural glucosinolates can act as prodrugs for novel ITCs, with a mechanism of action reliant on their hydrolytic cleavage by myrosinase. Such non-natural glucosinolates may serve as very economical chemopreventive agents for individuals at risk for cancers of and around the GI tract.

Introduction

Diets rich in fruits and vegetables are associated with reduced risks of degenerative diseases such as cancer and cardiovascular disease.[1,2] Crucifers such as broccoli, cauliflower, watercress, Brussels sprouts, and cabbage exhibit pronounced chemopreventive effects, many of which likely result from the bioactivities of organic isothiocyanates (ITCs) abundant in these plants.[3,4] Many ITCs have been shown to prevent chemical carcinogenesis by enhanced detoxification of reactive carcinogens through the induction of phase II enzymes such as glutathione *S*-transferases, NAD(P)H:quinone reductase, epoxide hydrolase, and UDP-glucuronosyl-transferases.[4–8] ITCs also block carcinogen activation by reducing expression levels of phase I enzymes and stimulating apoptosis of damaged cells.[9–12]

Although organic isothiocyanates were identified as the agents responsible for the chemopreventive and chemotherapeutic benefits of *Brassica* vegetables, they are not natively produced in the plants. Rather, isothiocyanates result from the enzymatic degradation of glucosinolate natural products (Scheme 1). Glucosinolates (β-thioglucoside-*N*-hydroxysulfates: e.g., glucoraphin **1**) are amino-acid-derived secondary metabolites that can be cleaved by the enzyme myrosinase (β-thioglucoside glucohydrolase, EC 3.2.3.1).[13–15] Myrosinase and glucosinolates are localized in separate plant cells—myrosin cells and S-cells, respectively—or in separate intracellular compartments.[16–19] Herbivore attack, particularly by chewing insects, causes tissue disruption, thereby bringing glucosinolates into contact with myrosinase.[14] In addition, intestinal bacteria produce myrosinase, which allows humans to degrade dietary glucosinolates even when plant myrosinase has been inactivated through cooking.[19,20] Myrosinase catalyzes the hydrolysis of the thioglucosidic linkages in glucosinolates to form unstable aglycone intermediates. Depending on the structure of the



Scheme 1. Metabolism of glucosinolates, exemplified by glucoraphanin. Deglycosylation of glucoraphanin (**1**) by myrosinase, followed by subsequent Lossen rearrangement, yields the isothiocyanate L-sulforaphane (**2**), which is in turn further metabolized through the mercapturic acid pathway to cysteine-conjugate **3**.

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aglycone side chain and the presence of additional proteins and cofactors, the aglycones degrade to a variety of bioactive products, including isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidine-2-thiones.[14,21–27] At physiological pH, these intermediates predominantly undergo Lossen rearrangements to form the corresponding isothiocyanates (such as **2**).[21,25,28] Importantly, intact glucosinolates themselves have no documented bioactivity.[29]

The specific reaction depicted in Scheme 1 is clinically relevant, as the isothiocyanate L-sulforaphane (L-SFN, **2**) is the principal inducer of phase II enzymes in broccoli extracts and is well documented as a potent chemopreventive agent.[30–33] L-SFN attenuates carcinogenesis at multiple stages through the Nrf2-dependent activation of the antioxidant response element (ARE).[34] Importantly, reduced expression of phase I enzymes and induction of phase II enzymes are attributed to activation of the ARE through thiocarbamoylation of the enzyme Keap1, which ordinarily exists in complexation with the ARE-targeted transcription factor Nrf2.[35] The majority of ARE inducers are electrophiles capable of modifying cysteine, suggesting that Keap1 cysteines are targeted by these compounds in signaling ARE induction.[36] Although modification of specific Keap1 cysteines by ARE inducers has been postulated to cause dissociation of the Keap1–Nrf2 complex directly, resulting in Nrf2 nuclear accumulation,[31,37] recent studies have indicated that Keap1 cysteine modification by **2** and other isothiocyanates is insufficient to disrupt the Keap1–Nrf2 interaction.[38]

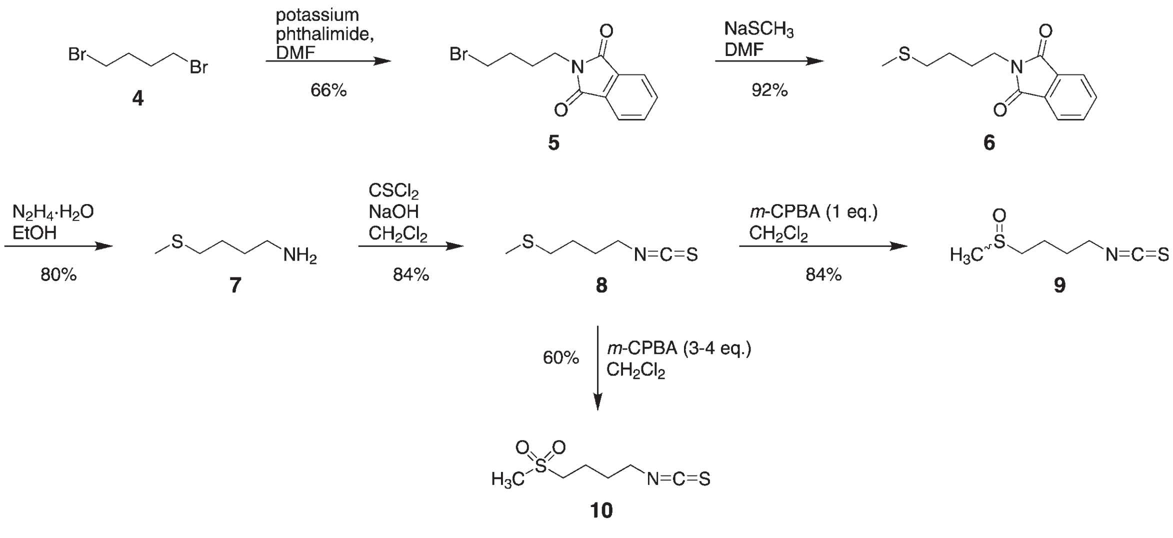
The structural identities of isothiocyanates appear to play a key role in the degree of elicited chemopreventive properties. Minor changes in isothiocyanate structure have been shown to impact in vitro bioactivities significantly.[4,39–43] These empirical findings may be explained, in part, by the observation that a primary metabolite of **2**, L-sulforaphane-L-cysteine (**3**), elicits moderate histone deacetylase (HDAC) inhibition.[44] Numerous studies on HDAC inhibitors have shown that similarly minor changes in structure can profoundly alter bioactivity.[45–50] Although a tremendous amount of structure–activity relationship data is now available for HDAC inhibitors,[51,52] significantly less information relating to those structural attributes of small molecules responsible for chemopreventive bioactivities is available. Although HDAC inhibition represents but one possible target of ITC action, we hypothesized that the structure of **2** is likely suboptimal for bioactivity and that novel isothiocyanates with enhanced chemopreventive properties relative to **2** could be identified. Moreover, we proposed that the documented activity of GI-tract-localized myrosinase could be exploitable to unmask relevant non-natural glucosinolates to afford their respective ITCs capable of eliciting enhanced anticarcinogenic properties. Non-natural glucosinolates that demonstrate GI-specific activation are likely to serve as important chemopreventive agents for those genetically predisposed to colorectal cancer and other cancers that target organs proximal to the GI tract.

We present here preliminary efforts toward the design of non-natural glucosinolates as novel prodrug chemopreventive agents. A library of synthetic isothiocyanates was constructed and bioactivities against tumor cells relative to those of L-SFN were comparatively analyzed. These studies resulted in the identification of multiple ITCs with improved potency and selectivity for cancerous cells relative to L-SFN, as well as several structure–activity trends relating to ITC cytotoxicity. Several ITCs were identified as lead compounds, and their corresponding non-natural glucosinolates were constructed. We have observed that these synthetic glucosinolates are substrates for myrosinase bioactivity, with enzymatic hydrolysis resulting in evolution of their respective ITCs. Together, these findings suggest the viability of exploiting myrosinase within the human GI tract to achieve drug specificity for organs in and around the GI tract.

Results and Discussion

Synthesis of D,L-sulforaphane

The syntheses of D,L-sulforaphane and erysolin were carried out as highlighted in Scheme 2. This overall procedure was

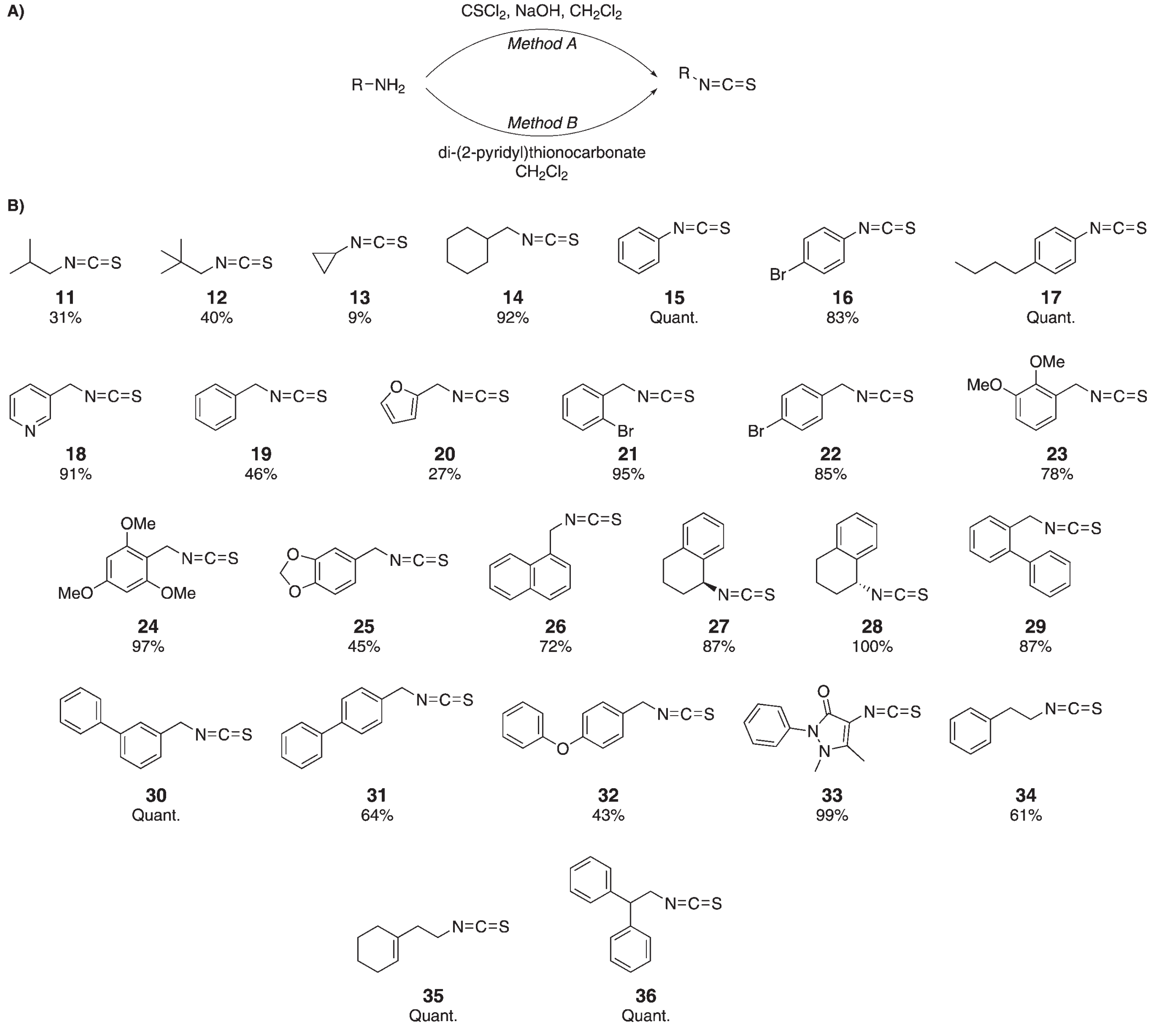


Scheme 2. Synthesis of D,L-sulforaphane (**9**) and erysolin (**10**).

modified from previously reported work by Vermeulen et al., both to increase yields and in order to obtain erysolin (**10**).[53] Specifically, an excess of 1,4-dibromobutane was used to form the single-displacement S_N2 product **5** predominantly upon addition of potassium phthalimide. The disubstituted product was the only significant side-product and **5** could readily be isolated by flash chromatography. Displacement of the remaining bromide in **5** was accomplished with a slight excess of sodium thiomethoxide. Trituration and the subsequent removal of residual water afforded **6** in consistently high yields. Unmasking of the phthalimide by treatment with hydrazine monohydrate under reflux conditions, followed by distillation, yielded **7** in 80% yield, a significant improvement over previous methods.[53] Importantly, we found that elimination of the acidic workup step and distillation of the oil **7** from the residual solid reaction byproduct greatly reduced the net loss of product. Treatment of **7** with an excess of thiophosgene under basic conditions yielded the isothiocyanate **8**. With **8** as a common intermediate, oxidation products **9** and **10** were obtained by using either stoichiometric or excess equivalents of *m*-CPBA. The published procedure that this synthetic effort was based upon reports a yield of 20% over five steps for **9**.[53] However, our modification of this procedure increases this yield to 34% over the same number of steps.

Syntheses of isothiocyanates

Utilizing generalized procedures for conversion of a primary amine into an isothiocyanate, we set out to construct a small library of isothiocyanates. Commercially available primary amines were selected for inclusion by a number of criteria, including steric volume, alkyl ring size, aromaticity, the number of methylene units, ring substitution patterns, conformational restriction, and bioisosteric substitution. Primary amines were treated with excesses of thiophosgene and isolated by standard column chromatography (Scheme 3 A).[53] Isothiocyanates



Scheme 3. Syntheses of isothiocyanates and panel of isothiocyanates screened. A) Synthesis of isothiocyanates. B) Isothiocyanates with yields.

11–**36** were obtained in yields ranging from 9% to quantitative (Scheme 3B). It was observed that isothiocyanates with low molecular weights and small alkyl chains typically had the lowest yields, likely a result of their increased volatility and loss during purification. Additionally, we hypothesize that certain functionalities present in the primary amines were not entirely stable to the harsh thiophosgene conditions. Repeated attempts to obtain **18** by using thiophosgene resulted in several unidentified breakdown products and a maximum yield of 14%. However, **18** could be obtained in 91% yield by employing a different isothiocyanate-installing reagent.[54] Utilization of di-(2-pyridyl)thionocarbonate rather than thiophosgene and sodium hydroxide offered a milder and less hazardous means to install isothiocyanates. Although this reagent is much more expensive than thiophosgene, we have observed that its general utility supercedes that of thiophosgene in nearly all regards excluding cost. Subjection of 3-picolylamine to di-(2-pyridyl)thionocarbonate readily provided **18**.

Cytotoxicity of isothiocyanates

The activity of isothiocyanate library members was assessed by two cytotoxicity assays in a total of eight human cancer cell lines representing a broad range of carcinomas, including breast, colon, CNS, liver, lung, ovary, prostate, and a mouse mammary normal epithelial control line (Figure 1). L-Sulforaphane (**2**) was found to be moderately cytotoxic, with an average IC_{50} of 5.7 ± 2.2 μM across all eight human cancer cell lines, but was nonspecific because it affected all cells, including NmuMG ($IC_{50} = 6.6 \pm 0.7$ μM), with similar efficiencies (Table 1). Five compounds that exhibited overall enhanced activities relative to L-SFN were identified from the isothiocyanate library. Library members **18** (average $IC_{50} = 2.5 \pm 1.0$ μM), **29** (average $IC_{50} = 4.7 \pm 1.2$ μM), **30** (average $IC_{50} = 3.3 \pm 1.1$ μM), **32** (average $IC_{50} = 4.8 \pm 1.6$ μM), and **36** (average $IC_{50} = 3.8 \pm 1.3$ μM) exhibited increased potency over L-SFN. This was especially evident for **18**, which was a highly potent cytotoxin against every human cancer cell line tested [1.2 ± 0.1 μM in the case of NCI/ADR RES, 7.5 times more potent than L-SFN (9.0 ± 0.7 μM)]. Additionally, **18** displayed moderate selectivity for cancerous cells over NmuMG (26.0 ± 1.2 μM, ~fourfold less potent than L-SFN). If both increased potency and selectivity are taken into account, these data indicate that **18** has nearly 30 times greater potential therapeutic effectiveness than L-SFN.

Isothiocyanates **29** and **32** are also notable for their enhanced selectivity toward cancerous cells over healthy cells, as indicated in Table 1. Even though compounds **29** and **32** both exhibit potencies against cancer cells comparable to that of **2**, they are significantly more selective for cancer cells over normal ones. While, on average, compound **2** displays only 1.3

Table 1. Range and mean IC_{50}s for isothiocyanates across eight human cancer cell lines and NmuMG control cells.

Compound	Low IC_{50} [μM][a]	High IC_{50} [μM][a]	Average carcinoma IC_{50} [μM][b]	IC_{50} (NmuMG) [μM][c]
2	3.1 ± 0.9 (MCF7)	9.0 ± 0.7 (NCI/ADR RES)	5.7 ± 2.2	6.6 ± 0.7
9	2.8 ± 0.1 (Hep3B)	16.5 ± 1.4 (NCI/ADR RES)	7.6 ± 4.2	3.2 ± 0.2
8	8.9 ± 0.4 (SF-268)	45.3 ± 4.9 (NCI-H460)	22.0 ± 11.9[d]	23.5
10	2.3 ± 0.8 (MCF7)	11.1 ± 0.6 (NCI/ADR RES)	5.6 ± 3.4	5.3 ± 0.4
11	27.6 ± 7.0 (HCT-116)	> 50.0 (multiple)	> 50.0[e]	> 200.0
12	30.3 (NCI-H460)	> 50.0 (multiple)	> 50.0	> 200.0
13	12.6 ± 0.8 (MCF7)	> 50.0 (multiple)	> 50.0[f]	> 200.0
14	15.4 ± 1.5 (SF-268)	> 50.0 (NCI/ADR RES)	29.3 ± 10.6[g]	14.8 ± 0.4
15	> 50.0 (multiple)	> 50.0 (multiple)	> 50.0	> 200.0
16	26.7 ± 4.9 (MCF7)	> 50.0 (multiple)	> 50.0[h]	21.5 ± 1.2
17	19.4 ± 0.8 (HCT-116)	> 50.0 (multiple)	35.2 ± 11.6[i]	65.7 ± 5.1
18	1.2 ± 0.1 (NCI/ADR RES)	4.5 ± 0.2 (NCI-H460)	2.5 ± 1.0	26.0 ± 1.2
19	3.2 ± 0.3 (HCT-116)	14.5 ± 1.2 (NCI-H460)	8.7 ± 3.7	15.4 ± 0.3
20	> 50.0 (multiple)	> 50.0 (multiple)	> 50.0	150.8 ± 29.7
21	2.7 ± 0.2 (MCF7)	20.1 ± 0.9 (NCI/ADR RES)	10.1 ± 6.8	4.6 ± 0.6
22	5.9 ± 0.6 (MCF7)	22.3 ± 1.6 (NCI/ADR RES)	10.7 ± 5.7	9.8 ± 0.7
23	3.2 ± 0.7 (MCF7)	16.1 ± 1.1 (NCI-H460)	6.8 ± 4.0	3.8 ± 0.3
24	3.2 ± 0.0 (MCF7)	15.2 ± 0.7 (NCI/ADR RES)	6.5 ± 4.5	2.1 ± 0.1
25	3.2 ± 0.7 (MCF7)	9.8 ± 0.5 (NCI/ADR RES)	5.8 ± 2.6	17.0 ± 1.1
26	2.0 ± 0.2 (SF-268)	19.8 ± 1.0 (Hep3B)	9.2 ± 6.8	21.5 ± 1.2
27	10.5 ± 0.7 (SF-268)	30.9 ± 2.8 (NCI/ADR RES)	17.9 ± 7.1	15.4 ± 0.3
28	3.6 ± 0.2 (Hep3B)	13.3 ± 1.4 (NCI-H460)	8.3 ± 2.8[j]	17.0 ± 1.1
29	3.2 ± 0.3 (Du145)	6.4 ± 0.2 (NCI-H460)	4.7 ± 1.2	180.1
30	2.1 ± 0.0 (Hep3B, MCF7)	5.3 ± 0.2 (NCI-H460)	3.3 ± 1.1	3.1 ± 0.4
31	4.4 ± 0.1 (SF-268)	15.0 ± 1.5 (NCI-H460)	7.9 ± 4.2	65.7 ± 5.1
32	3.3 ± 0.2 (NCI/ADR RES)	8.2 ± 0.4 (Du145)	4.8 ± 1.6	186.6
33	> 50.0 (multiple)	> 50.0 (multiple)	> 50.0	> 200.0
34	7.2 ± 0.4 (SF-268)	13.7 ± 0.5 (NCI-H460)	11.5 ± 2.1	17.7 ± 1.0
35	3.9 ± 0.5 (SF-268)	28.6 ± 3.4 (NCI-H460)	18.3 ± 8.0	13.0 ± 0.7
36	1.2 ± 0.0 (HCT-116)	5.6 ± 0.4 (NCI-H460)	3.8 ± 1.3	3.6 ± 0.3

[a] ± SEM (cell line). [b] ± Standard deviation (cell line). [c] ± SEM. [d] $IC_{50} > 30$ μM in NCI/ADR RES. [e] $IC_{50} = 27.6$ μM in HCT-116, 32.8 μM in Hep3B. [f] $IC_{50} = 12.6$ μM in MCF7. [g] Noninhibitory in NCI/ADR RES. [h] Calculated solely from Calcein AM data. [i] Noninhibitory in Hep3B, NCI/ADR RES, NCI-H460. [j] No data in NCI/ADR RES

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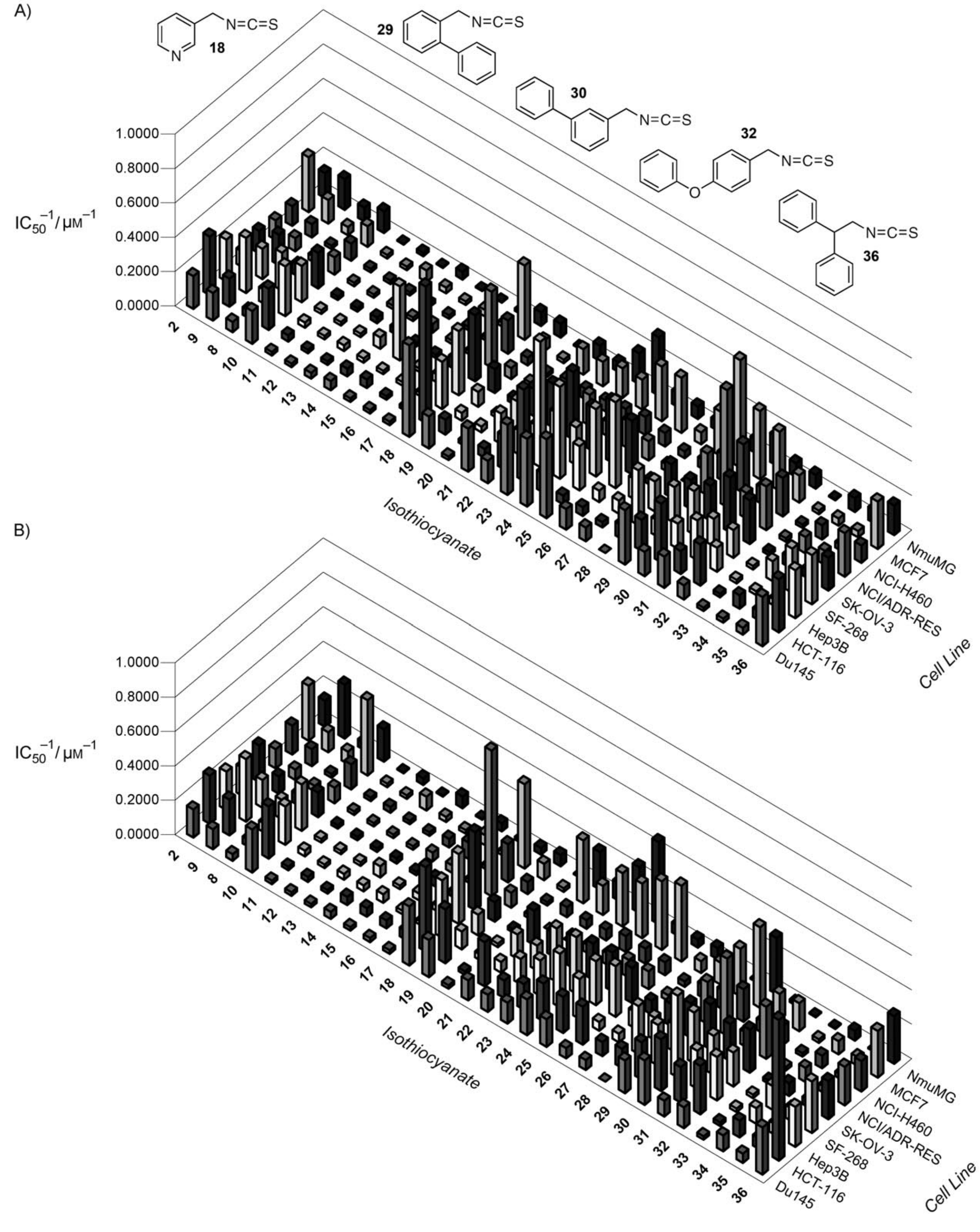
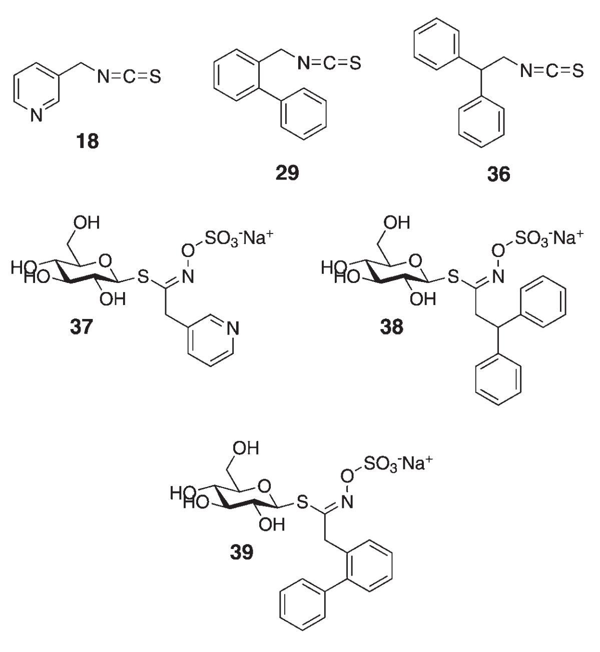


Figure 1. Summary of the IC$_{50}$ data from Calcein AM and CellTiter-Glo high-throughput cytotoxicity assays. Reciprocal IC$_{50}$ values are displayed for clarity, with the current figure representing an IC$_{50}$ range of 1.2 μM (**18**, NCI/ADR RES) to $>$50.0 μM (e.g., **11**, all cell lines). Compounds exhibiting IC$_{50}$s greater than 50.0 μM were considered to be non-inhibitory (IC$_{50}^{-1}=0$) in all cell lines, with the exception of the NmuMG, where 200.0 μM was used. The IC$_{50}$ value for each library member represents at least three replicates of dose-response experiments conducted over five concentrations at twofold dilutions. IC$_{50}$ values and corresponding error values can be found in the Supporting Information. The five library member "hits" are shown for structural comparison. A) Reciprocal IC$_{50}$s calculated by the Calcein AM assay. Live cells were distinguished by the presence of a ubiquitous intracellular enzymatic activity that converts the nonfluorescent, cell-permeable molecule calcein AM into the intensely fluorescent molecule calcein, which is retained within live cells. B) Reciprocal IC$_{50}$s calculated by the CellTiter-Glo assay. Live cells were observed by fluorescence through the enzymatic action of luciferase on luciferin, a process dependent on and proportional to the cellular concentration of ATP. Du145: human prostate carcinoma; HCT-116: human colon carcinoma; Hep3B: human liver carcinoma; SF-268: human CNS glioblastoma; SK-OV-3: human ovary adenocarcinoma; NCI/ADR RES: human breast carcinoma; NCI-H460: human breast carcinoma; MCF7: human breast carcinoma; NmuMG: mouse mammary normal epithelial cells.

times the activity against cancer cells relative to NmuMG cells, **29** and **32** are 36.3 and 38.6 times more selective, respectively. This level of selectivity far surpasses what was exhibited by other members of the isothiocyanate library and appears to be highly dependent on the precise substitution pattern of the parent isothiocyanate. Comparison of **29**, **30**, and **31** indicates that significant cell selectivity is only observed for (biphenyl-2-yl)methyl **29** (36.3-fold) and (biphenyl-4-yl)methyl **31** (8.3-fold); (biphenyl-3-yl)methyl **30** is unselective.

From the results of these cytotoxicity assays, three synthetic isothiocyanates were selected as candidates for the construction of the corresponding non-natural glucosinolates; the structures of these isothiocyanates and glucosinolates are depicted in Scheme 4. Each of these compounds was found to exhibit differentially enhanced bioactivities relative to the lead compound L-sulforaphane (**2**). 3-(Pyridylmethyl) isothiocyanate (**18**) was selected as the single most potent isothiocyanate screened, with an observed average IC_{50} of 2.5 ± 1.0 μM. (Biphenyl-2-yl)methyl isothiocyanate (**29**) was chosen for its profound selectivity for cancer cells over NmuMG cells, in addition to its enhanced potency relative to **2**. Finally, 2,2-diphenylethyl isothiocyanate (**36**) was selected for its potency in relation to **2** as well as its amenability toward extremely accurate reproducibility. With these aglycone structures, non-natural glucosinolates **37**, **38**, and **39** were identified as attractive synthetic targets.

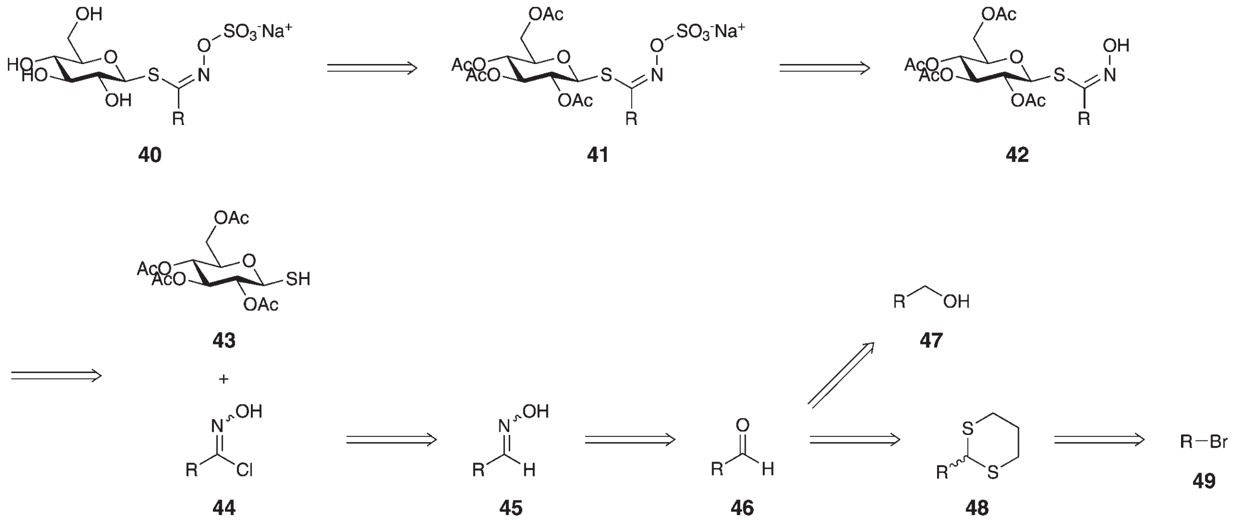


Scheme 4. Lead isothiocyanates and their corresponding target non-natural glucosinolates.

Syntheses of non-natural glucosinolates

Although glucosinolates are often isolated directly from plant tissue,[55,56] many of the naturally occurring ones have been synthesized.[57,58] In addition, the syntheses of many different glucosinolate analogues have also been reported, including analogues containing non-natural aglycones, α-glucosinolates, sugar variants, deoxy 1-thio-glucose derivatives, aza-desulfoglucosinolates, phosphate bio-isosteres, fluorinated analogues, and *C*-glucosinolates.[59–66] The synthetic pathways that have been developed for glucosinolates over recent decades are invariably based on a key coupling step between partially protected 1-thio-β-D-glucopyranose and a highly reactive hydroximoyl chloride to yield a (*Z*)-thiohydroximate precursor.[67]

In general, we hoped to apply these preexisting synthetic strategies toward the construction of glucosinolates **37**, **38**, and **39**. Our retrosynthetic analysis was largely based upon previously published routes (Scheme 5).[68] The strategy envisioned was intended to be amenable to a wide assortment of targets and thus diversity-oriented, given our long-term interest in developing myrosinase-activated bioactive substances. Removal of acetyl groups was envisioned as the final step in the synthesis of **40**. The sulfate precursor **41** would be gener-



Scheme 5. General retrosynthesis for glucosinolates **37**, **38**, and **39**.

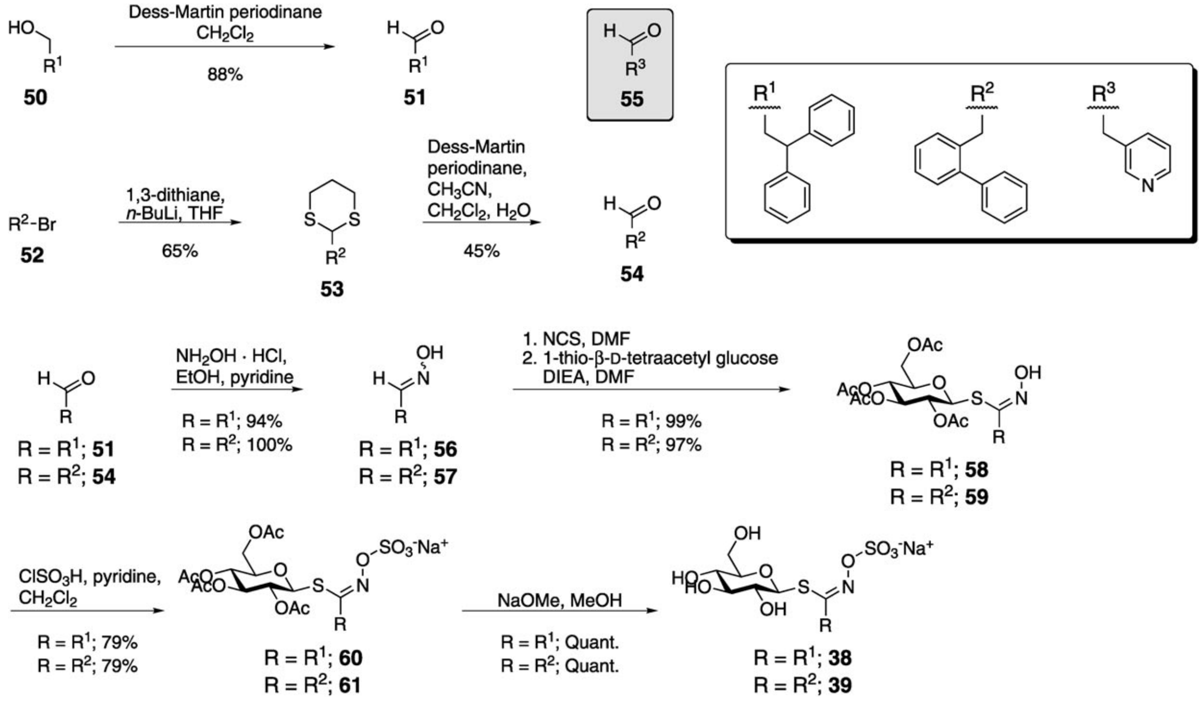
ated through selective sulfation of the thiohydroximate. Compound **42** could be formed through the coupling of commercially available 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucose (**43**) to various hydroximoyl chlorides **44**. These hydroximoyl chlorides could be generated through chlorination of oximes **45**, which in turn could be derived from aldehydes **46**. We believed that aldehyde **46** was the crucial synthon. The aldehyde precursors of glucosinolates **37** and **38** would be generated by hydrolysis of 1,3-dithianes **48**, which in turn could be formed through treatment of 1,3-dithiane carbanion with their corresponding commercially available alkyl bromides **49**. Alternatively, the aldehyde precursor of glucosinolate **38** would be readily generated through simple oxidation of commercially available primary alcohol **47**.

The synthesis of appropriate aldehydes from commercially available materials was envisioned as the initial step in the generation of non-natural glucosinolates. As shown in Scheme 6, aldehyde **51** was obtained in 88% yield through the oxidation of **50** with Dess–Martin periodinane.[69] In contrast, alkylation of (biphenyl-2-yl)methyl bromide (**52**) with 1,3-dithiane anion produced **53** in moderate yield.[70] Dethioacetylization of **53** by treatment with Dess–Martin periodinane resulted in aldehyde **54**.[71] Although we have shown that **54** can be synthesized by this route, the yield was found to be only 29% over two steps; ideally, this could be improved upon through future optimization. Unfortunately, aldehyde **55** was not a viable synthetic target en route to glucosinolate **37** (data not shown). This was not surprising, given that we could find only one account of **55**'s synthesis in the literature, and the pyridyl nitrogen clearly represents a reactive liability.[72]

The aldehyde functionality in **51** and **54** provided a convergent route to glucosinolates **38** and **39**. Aldehydes **51** and **54** were transformed into their corresponding oximes **56** and **57** through treatment with hydroxylamine in exceptionally high yield, as a 1.8:1 and a 1.2:1 ratio of isomers, respectively.[68] Crude **56** and **57** exhibited greater than 95% purity by ^{1}H NMR and were used without purification. Literature precedent has shown that hydroximoyl chlorides can be derived from oximes by treatment with *N*-chlorosuccinimide.[68,73,74] However, the hydroximoyl chlorides of **56** and **57** proved to be highly unstable and not amenable to purification. To circumvent this issue, hydroximoyl chlorides were generated in situ by treatment of **56** or **57** with *N*-chlorosuccinimide in DMF.[68] Subsequent addition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucose and DIEA afforded **58** and **59**, respectively, in high yields and as single isomers (99% and 97%, respectively). Sulfation of the thiohydroximate in **58** and **59** was accomplished with chlorosulfonic acid and pyridine; the resulting sodium salts were isolated by silica gel chromatography (79% yields). Zemplen de-*O*-acetylations of **60** and **61** afforded the final deprotected glucosinolates **38** and **39** in quantitative yields. Overall, **38** was synthesized in 65% yield over five steps, while **39** was synthesized in 22% yield over six steps. It must be noted that the yield of **39** is most significantly attenuated by the first two steps; production of aldehyde **54** is particularly low-yielding.

Myrosinase-catalyzed glucosinolate hydrolysis

Myrosinase specific activity was determined spectrophotometrically at 227 nm with sinigrin **62** as substrate according to the established protocol, where one myrosinase unit was defined as the amount of enzyme able to hydrolyze 1 nmol **62** min^{-1} at pH 7.4 and 37 °C.[75] Lineweaver–Burk analysis for the enzymatic hydrolysis of **62** was also performed by the same



Scheme 6. Syntheses of non-natural glucosinolates **38** and **39**.

method, and K_m and V_{max} values were determined to be 117 μM and 1.10 U μL^{-1}, respectively (Figure 2). This determined K_m in particular agrees closely with the documented K_m of 115 μM.[76] Unfortunately, the hydrolysis of **38** and **39** could not be accurately determined by this method, likely as a result of complications due to the limited solubility of hydrolysis products in aqueous buffer (data not shown). Although ITC solubility could be reestablished with the addition of CH_3CN, we observed that this resulted in the complete loss of myrosinase activity. Given these limitations, hydrolysis reactions were instead monitored by reversed-phase HPLC (RP-HPLC).

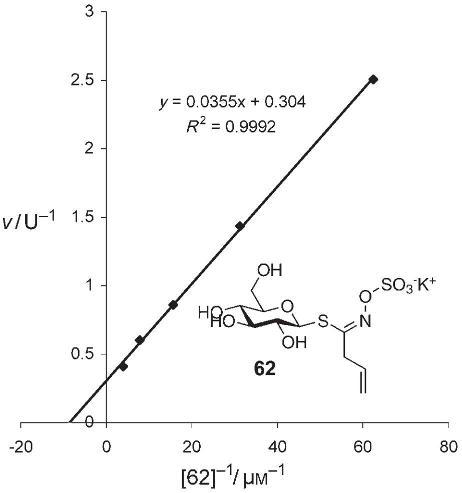


Figure 2. Lineweaver–Burk plot for the hydrolysis of sinigrin (**62**) by myrosinase. Rates were determined from data from the initial 3 min of spectrophotometric assay in a total volume of 1.000 mL containing 2.74 U myrosinase. Calculated values for **62**: $K_m = 117$ μM, $V_{max} = 1.10$ U μL^{-1} enzyme stock.

RP-HPLC method development was largely based on a published separation method for multiple organic isothiocyanates.[77] Standards of glucosinolates **62**, **38**, and **39**, isothiocyanates **29** and **36**, and the corresponding primary amines 2,2-diphenylethylamine (**63**) and (biphenyl-2-yl)methyl amine (**64**) were characterized (Table 2). All compounds exhibited broad elution peaks giving homogeneous UV-visible spectra at all timepoints, implicating that the broadening was characteristic of the compounds and/or the HPLC system. Injections of greater than 20 nmol for glucosinolates **38** and **39** in CH_3CN/phosphate buffer (0.1 M, pH 7.4) 1:1 were especially prone to overloading and partial elution in the void volume. For each compound, the relationship between the amount of compound injected and baseline-corrected peak area was linear between 5.0 and 50.0 nmol, with linear correlation coefficients (r^2) ranging from 0.9923 to 0.9993. For mixed standards containing **36**/**38** and **29**/**39**, elution peaks of the corresponding glucosinolate/isothiocyanate pairs were completely resolved by more than 10 min. In addition, the areas of integration for all peaks were indistinguishable from standards obtained by injection of single compounds. Addition of 50% (v/v) acetonitrile to aqueous solutions of **29** and **36** resulted in the complete restoration of detectable isothiocyanate and strongly suggested that this method would provide a rapid means by which to monitor hydrolysis reactions performed solely in phosphate buffer (Figure 3).

Table 2. Spectral characterization of isothiocyanates, glucosinolates and amines by RP-HPLC injection.

Compound	Solvent[a]	t_R [min]	λ_{max} [nm]
29	A	41.0–56.0	234.4
36	A	40.0–54.0	217.9,[b] 246.1
38	B	16.0–28.0	219.1[b]
	A	0.6–1.8, 8.0–28.0	219.1[b]
39	B	15.0–27.0	234.4
	A	0.6–1.8, 8.0–28.0	234.4
62	B	0.6–2.0	227.3
63	A	16.5–30.0	216.7,[b] 258.0
64	A	15.0–30.0	234.4

[a] Solvent A: 1:1 CH_3CN/phosphate buffer (0.1 M, pH 7.4); solvent B: phosphate buffer (0.1 M, pH 7.4). [b] First derivative λ_{max}

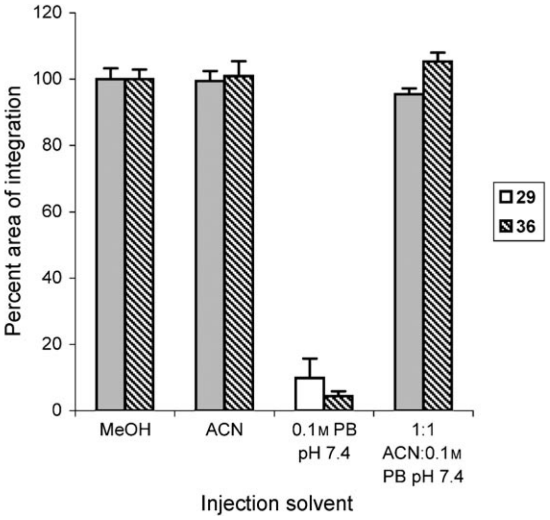


Figure 3. Relative RP-HPLC integration areas for isothiocyanates in injection solvents. All isothiocyanate solutions were 1.0 mM and independently constructed in triplicate. Areas of integration for isothiocyanate elution peaks were calculated, averaged, and standardized against those obtained by using MeOH. Error bars represent standard deviation ($n = 3$). The addition of CH_3CN (50% v/v) to solutions of **29** and **36** in aqueous buffer restored the solubility to levels observed solely in organic solvents. CAN: acetonitrile. PB: phosphate buffer (0.1 M, pH 7.4).

Hydrolysis reactions for **38**, **39**, and **62** were performed in phosphate buffer (0.1 M, pH 7.4) at 37 °C and monitored by RP-HPLC. For all glucosinolates tested, we observed time-dependent decreases in concentration over 24 h, as monitored by the standardized integrated peak area, height, and also the cross-sectional UV-visible spectra (Figure 4). We found that this effect was myrosinase-dependent; control reaction mixtures incubated at 37 °C but lacking myrosinase did not show appreciable decreases in glucosinolate concentration after 24 h (Figure 5). Importantly, reactions using **38** and **39** showed an increase in peak signal between 40–56 min as a function of

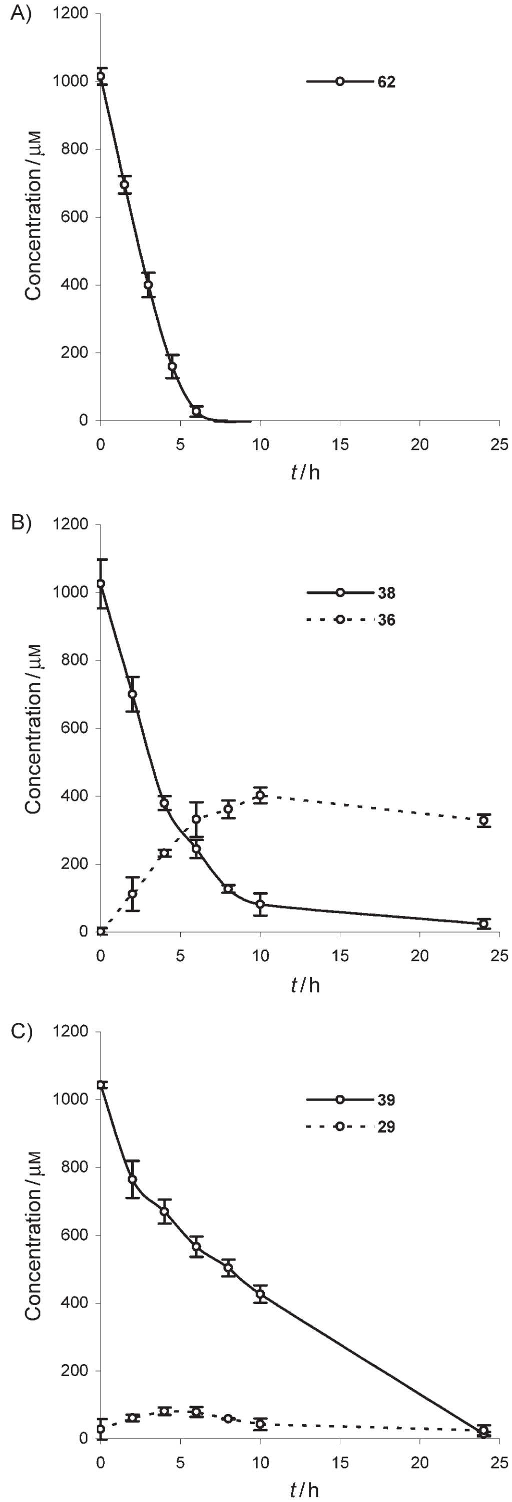


Figure 4. Summary of hydrolysis reactions of glucosinolates with myrosinase. Initial concentrations of glucosinolates were 1 mм in phosphate buffer (0.1 м, pH 7.4). Reactions were analyzed by RP-HPLC, and concentrations of glucosinolates and isothiocyanates were calculated by use of standard curves. Error bars represent the standard deviation ($n=3$). A) Hydrolysis of sinigrin (**62**, 20 µg myrosinase mL^{-1} buffer). The formation of allyl isothiocyanate was not determined. B) Hydrolysis of 2,2-diphenylethyl glucosinolate **38** to 2,2-diphenylethyl isothiocyanate (**36**, 64 µg myrosinase mL^{-1} buffer). C) Hydrolysis of (biphenyl-2-yl)methyl glucosinolate **39** to (biphenyl-2-yl)-methyl isothiocyanate (**29**, 160 µg myrosinase mL^{-1} buffer).

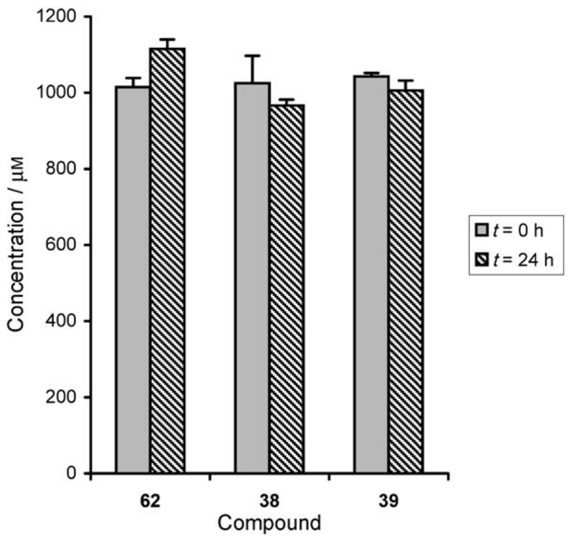


Figure 5. Stability of glucosinolates in phosphate buffer (0.1 м, pH 7.4) at 37 °C. Prolonged exposure to reaction conditions without the addition of myrosinase did not result in detectable decreases in glucosinolate concentration over time.

time. Both the cross-sectional UV-visible spectra and the retention times of these peaks matched the corresponding standards for isothiocyanates **29** and **36** (see Table 2). In addition, UV-visible spectra were uniform across the broad elution peaks, implicating that these peaks each represented a single eluted compound. Taken together, these observations strongly suggest that these newly formed peaks correlate to isothiocyanates **29** and **36**.

Hydrolysis experiments were repeated in triplicate, and concentrations of all glucosinolates and isothiocyanates were calculated by use of standard curves. The mean concentrations and standard deviations at each reaction timepoint were calculated (Figure 4). In general, glucosinolate and isothiocyanate concentrations across replicates exhibited excellent precision, with standard deviations ($n=3$) ranging from 0.06 to 72 µм. The rates of glucosinolate hydrolysis were calculated for **62**, **38**, and **39** by linear regression of average concentrations (Table 3). Glucosinolate **62** was hydrolyzed the most rapidly by

Table 3. Comparison of myrosinase activity determined for glucosinolates **62**, **38**, and **39**.

Glucosinolate	Rate of hydrolysis [$\mu M\,h^{-1}$][a]	V_{max} [$nmol\,min^{-1}\,mg^{-1}$]	k_{cat} [min^{-1}][b]	Relative activity [%]
62	190.6[c]	158.9	10.72	100.0
38	161.4[d]	42.0	2.84	26.5
39	42.1[e]	4.4	0.30	2.8

[a] Calculated from linear regression of averages for **62** (0–6 h), **38** (0–6 h), and **39** (2–10 h) and representing initial reaction velocities. [b] Calculated with a dimeric molecular weight of 135 kDa and the assumption that there are two active sites per dimer. [c] Rate with 20.0 µg myrosinase mL^{-1} buffer. [d] Rate with 64.0 µg myrosinase mL^{-1} buffer. [e] Rate with 160.0 µg myrosinase per mL buffer.

myrosinase, with a maximum velocity (V_{max}) of 158.9 nmol min^{-1} per mg protein. In contrast, glucosinolates **38** and **39** were found to have V_{max} values of 42.0 and 4.4 nmol min^{-1} per mg protein. Enzymatic turnover rates (k_{cat}) were calculated from the reported average dimeric molecular weight of 135 kDa from myrosinase in *Sinapis alba* and on the assumption that there are two active sites per dimer.[78,79] Glucosinolates **62**, **38**, and **39** showed k_{cat} values of 10.72, 2.84, and 0.30 min^{-1}, respectively. Importantly, non-natural glucosinolates **38** and **39** were found to exhibit 26.5% and 2.8% relative activities, respectively, in comparison to the natural substrate sinigrin (**62**). In comparison, benzyl glucosinolate (glucotropaeolin) has been shown to have 63.3% of the relative activity of **62** in the presence of myrosinase isolated from *Brevicoryne brassicae*.[80] Given that glucosinolates **38** and **39** are both significantly larger than benzyl glucosinolate and are more likely to experience space restrictions within the enzyme active site, it is not entirely surprising that these compounds act as less effective myrosinase substrates than benzyl glucosinolate in comparison with **62**. This is especially evident for **39**, containing the highly rigid biaryl functionality. Overall, these data provide evidence that non-natural glucosinolates **38** and **39** are capable of hydrolysis by myrosinase, albeit with differing efficiencies.

In addition to providing relative rates of glucosinolate hydrolysis, the data in Figure 4 highlight the production of isothiocyanates **29** and **36** as the only observable hydrolysis products. Under these conditions, the hydrolysis of **38** resulted in the formation of isothiocyanate **36** with a maximum yield of approximately 40%, occurring at a time of 10 h. Conversely, isothiocyanate **29** was produced with a maximum average yield of 8% after 4 h. In similar studies, isothiocyanates have been reported in yields ranging from 60% to quantitative, with other hydrolysis products making up the remainder.[77,81] By virtue either of our detection method or enzymatic chemistry, we failed to observe signals indicative of hydrolysis products other than the expected isothiocyanates.

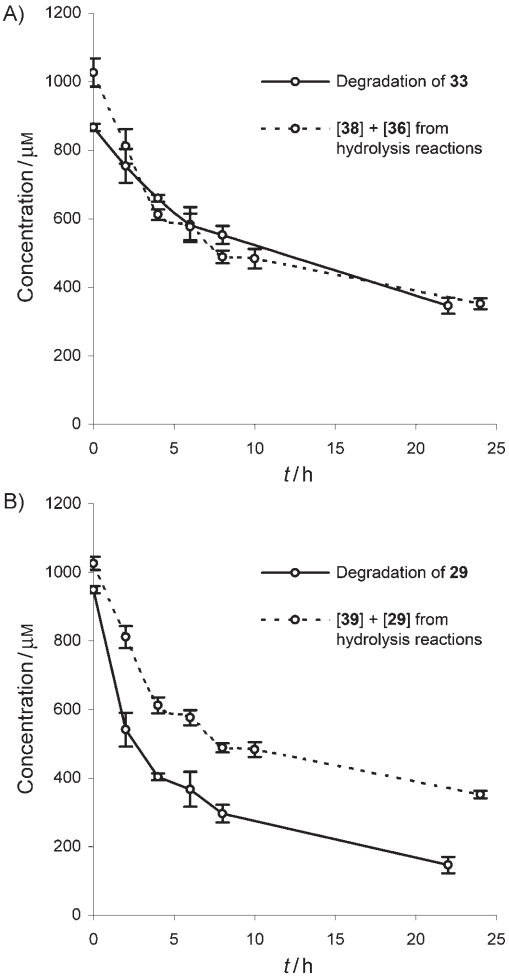


Figure 6. Stabilities of isothiocyanates **33** and **29** in phosphate buffer (0.1 M, pH 7.4) at 37 °C. Initial concentrations of isothiocyanates were 1 mM. Reactions were analyzed by RP-HPLC and isothiocyanate concentrations were calculated by use of standard curves. Error bars represent the standard deviation ($n = 3$). Dotted lines represent the sums of average glucosinolate and isothiocyanate concentrations at each timepoint from hydrolysis reactions, with error bars corresponding to the average standard deviation for added data points. A) Degradation of 2,2-diphenylethyl isothiocyanate (**36**). B) Degradation of (biphenyl-2-yl)methyl isothiocyanate (**29**).

Aqueous degradation of isothiocyanates

In reactions with both **38** and **39**, isothiocyanate concentrations declined over time after reaching their maxima, similarly to previous reports on the degradation of isothiocyanates in water.[25,77,81,82] In contrast to such cases in which the major degradation product was primarily the corresponding amine, neither **63** or **64** were detected in our reactions (see Table 2). Rather, isothiocyanates appeared simply to diminish over time, without explanation as to the factors responsible for this behavior or the final state of the isothiocyanates.

To allow better understanding of this phenomenon, the stabilities of isothiocyanates **29** and **36** in aqueous buffer were assessed by RP-HPLC. These assays were performed with initial isothiocyanate concentrations of 1 mM and with use of the same procedure and variables as in the hydrolysis experiments, only without the addition of myrosinase. Assays were performed in triplicate for each timepoint, and the average concentrations were plotted (Figure 6). Compounds **29** and **36** each exhibited a reproducible, time-dependent decrease in isothiocyanate concentration under the experimental conditions, albeit to varying degrees. The concentration of isothiocyanate **36** decreased at an average rate of 47 μM h^{-1} (11.8 nmol h^{-1}) over the first 8 h, with a slightly reduced rate of loss over the next 14 h. Conversely, **29** degraded much more quickly over the initial 4 h, with a rate of 136 μM h^{-1} (34 nmol h^{-1}), while the final 18 h showed a rate of loss similar to that observed with **36**. Both sets of data were compared with the time-dependent sums of the corresponding average glucosinolate and isothiocyanate concentrations from hydrolysis reactions (Figure 6, dotted lines), representing the detectable mass balances of the hydrolysis reactions, which theoretically should total the starting glucosinolate concentration of 1000 μM at all reaction timepoints. The observed rate of degeneration of **36** closely resembles the net loss in detectable compounds from hydrolysis reactions. Although the corresponding curves for the **29** system differed in absolute value by up to 20%, their line shapes and rates of loss were highly congruent. In short, these data suggest that the instability of isothiocyanates **29** and **36** under in vitro enzymatic conditions is a major impediment to their detection in myrosinase hydrolysis reactions of **38** and **39**. However, the magnitude of this effect may be substantially altered when performed in vivo, as isothiocyanates are likely to be readily absorbed by cells as they are produced within the GI tract.

Conclusions

We have synthesized a small library of ITCs and have identified several with increased cytotoxicity and selectivity over L-SFN in eight human cancer cell lines. Cytotoxicity assays also provided the means by which to identify isothiocyanates **18**, **29**, and **36** as lead compounds for the construction of the corresponding non-natural glucosinolates. Two of these target glucosinolates, **38** and **39**, were successfully constructed. We have developed an efficient RP-HPLC method to monitor the hydrolysis of glucosinolate substrates by myrosinase. This methodology provides highly reproducible results and offers several advantages over other documented techniques. In addition, it is also the first documented method for the generalized time-dependent, concomitant detection of glucosinolate/isothiocyanate pairs. Using this assay, we have shown that non-natural glucosinolates **38** and **39** are both substrates for myrosinase and that their enzyme-mediated hydrolysis results in the production of bioactive isothiocyanates **36** and **29**, respectively. Enzymatic hydrolysis of glucosinolate **38** proceeded with 26.5% of the efficiency of that of sinigrin **62**, resulting in a 40% yield of isothiocyanate **36**. We have also provided preliminary evidence that the observed limited yields of isothiocyanates are likely a result of their extended exposure to aqueous media at elevated temperatures. Although the instability of isothiocyanates in aqueous media has been well documented, we believe that these specific results may be an artifact of the artificially prolonged assay conditions required to obtain meaningful, time-dependent RP-HPLC analysis.

Although the in vitro enzymatic release of isothiocyanates from non-natural glucosinolates described is slower than the rates reported for their natural counterparts, this does not detract from the larger goal of developing new myrosinase-activated agents. For one thing, simply because in vitro enzymatic rates differ does not necessarily mean that in vivo rates will differ with similar magnitudes or even with the same relative rank. Secondly, the slower rates of myrosinase-catalyzed glucosinolate consumption may be compensated for (in vivo) both by continual dietary intake, and hence constitutive GI exposure, as well as by the absence or minimized toxicity of the excess, nonhydrolyzed glucosinolates. In addition, each of the released non-natural isothiocyanates exhibits enhanced potency relative to L-sulforaphane. Finally, it should be noted that the non-natural glucosinolates **38** and **39** are only first-generation analogues exhibiting the proof of principle; continued structure–activity studies are likely to identify analogues with increased potency and rates of enzymatic isothiocyanate release.

As a whole, this work forms a preliminary body of evidence supporting the use of synthetic glucosinolates as prodrugs for isothiocyanates known to exhibit chemopreventive and chemotherapeutic activities. Activation of these non-natural glucosinolates remains reliant on the same myrosinase-dependent mechanism as observed with glucosinolates found in many dietary vegetables. This is particularly significant as myrosinase is localized in humans to the GI tract and may be capable of similarly activating orally administered non-natural glucosinolates. Exploitation of myrosinase-dependent mechanisms of drug activation has not been extensively explored. We believe that these initial findings suggest that the natural biological activities of GI-tract-localized myrosinase could be harnessed to achieve drug specificity for organs in and around the GI tract.

Experimental Section

All reactions were carried out under argon unless indicated otherwise. All reagents were obtained from available commercial sources and were used without further purification unless otherwise noted. The silica gel used in column flash chromatography was 60 Å, 230–400 mesh. Analytical TLC was performed on EM Science silica gel plates with detection by UV light. NMR spectra were acquired on Varian Unity Inova 400 MHz and 500 MHz spectrometers with TMS or solvent as internal reference; the chemical shifts are reported in ppm, in δ units. Mass spectroscopic data were obtained at the University of Wisconsin-Madison Department of Chemistry or School of Pharmacy Analytical Instrumentation Centers.

2-(4-Bromobutyl)isoindoline-1,3-dione (5): A solution of 1,4-dibromobutane (4.40 mL, 36.46 mmol) in anhydrous DMF (52 mL) was chilled to 0 °C. After 15 min, potassium phthalimide (3.46 g, 18.67 mmol) was slowly added to the stirring solution, and the reaction mixture was allowed to warm to ambient temperature over 18 h. The mixture was concentrated in vacuo and co-stripped several times with anhydrous THF. Products were dissolved in H_2O/EtOAc (1:1, 200 mL) and the aqueous phase was extracted with EtOAc (3×100 mL). Combined organics were washed with brine, dried over Na_2SO_4, and filtered through a celite plug prior to concentration in vacuo. Silica gel chromatography (hexane/EtOAc 3:1) and subsequent concentration afforded **5** (3.47 g, 66%) as a white solid. ^{1}H NMR ($CDCl_3$): $\delta = 7.85$ (dd, $J = 5.4$, 3.1 Hz, 2H), 7.73 (dd, $J = 5.4$, 3.0 Hz, 2H), 3.73 (t, $J = 6.7$ Hz, 2H), 3.45 (t, $J = 6.4$ Hz, 2H), 1.89 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta = 168.5$, 134.1, 132.2, 123.4, 37.1, 32.9, 30.0, 27.4 ppm; HRMS (ESI): m/z: calcd for $[M+Na]^+$: 303.9949; found: 303.9936.

2-[4-(Methylthio)butyl]isoindoline-1,3-dione (6): Sodium thiomethoxide (3.81 g, 54.33 mmol) was dissolved in anhydrous DMF (40 mL), and the system was chilled to 0 °C. To this was added a solution of **5** (13.70 g, 48.56 mmol) in anhydrous DMF (95 mL). After 15 min at 0 °C, the reaction mixture was allowed to warm to ambient temperature over 18 h. The resulting solution was slowly poured into a stirring, ice-chilled bath of deionized water (800 mL). The precipitate was collected by filtration, washed with cold water, and redissolved in CH_2Cl_2 (400 mL). Organics were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to afford **6** (11.1 g, 92%) as pinkish-white crystals. ^{1}H NMR ($CDCl_3$): $\delta = 7.84$ (dd, $J = 5.4$, 3.1 Hz, 2H), 7.72 (dd, $J = 5.4$, 3.0 Hz, 2H), 3.71 (t, $J =$

7.1 Hz, 2H), 2.54 (t, $J=7.3$ Hz, 2H), 2.09 (s, 3H), 1.80 (m, 2H), 1.65 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): $\delta=168.5$, 134.0, 132.2, 123.3, 37.6, 33.7, 27.8, 26.5, 15.6 ppm; HRMS (ESI-EMM): *m/z*: calcd for $[M+Na]^+$: 272.0721; found: 272.0727.

4-(Methylthio)butan-1-amine (7): Hydrazine monohydrate (520 μL, 537 mg, 10.73 mmol) was added to a solution of **6** (2.00 g, 8.04 mmol) in absolute EtOH (48 mL). This solution was heated to reflux for 3 h and then cooled to 0 °C to precipitate the solid fully. The solid was removed by filtration and was washed excessively with anhydrous Et_2O (1 L). The filtrates were combined and concentrated in vacuo. Distillation at reduced pressure (6 mmHg, b.p. 55 °C) afforded **7** (762 mg, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$): $\delta=2.72$ (t, $J=6.7$ Hz, 2H), 2.52 (t, $J=7.4$ Hz, 2H), 2.10 (s, 3H), 1.64 (m, 2H), 1.55 (m, 2H), 1.33 ppm (brs, 2H); ^{13}C NMR ($CDCl_3$): $\delta=$ 42.1, 34.4, 33.2, 26.7, 15.7 ppm; LRMS (ESI): *m/z*: calcd for $[M+H]^+$: 120.1; found: 120.1.

1-Isothiocyanato-4-(methylthio)butane (8): Thiophosgene (1.38 mL, 18.10 mmol) was dissolved in anhydrous CH_2Cl_2 (41 mL), and the mixture was chilled to 0 °C. Compound **7** (698 mg, 5.86 mmol) and NaOH (607 mg, 15.17 mmol) were added in sequence, and the solution was allowed to warm to ambient temperature over 3.5 h. Solvents were mostly removed in vacuo and the remainder was filtered through celite to remove any solid. Silica gel chromatography (hexane/CH_2Cl_2 3:1) and subsequent concentration afforded **8** (795 mg, 84%) as an orange oil. ^{1}H NMR ($CDCl_3$): $\delta=3.55$ (t, $J=6.4$ Hz, 2H), 2.53 (t, $J=6.9$ Hz, 2H), 2.09 (s, 3H), 1.87–1.68 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta=129.4$, 44.4, 32.8, 28.5, 25.4, 14.9 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 161.0333; found: 161.0337.

1-Isothiocyanato-4-(methylsulfinyl)butane (9): A solution of *m*-CPBA (934 mg, 5.41 mmol) in anhydrous CH_2Cl_2 (6.25 mL) was slowly added to a solution of **8** (795 mg, 4.93 mmol) in anhydrous CH_2Cl_2 (7.0 mL). After 2 h of stirring at ambient temperature, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and the organics were washed with satd. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. Silica gel chromatography (CH_2Cl_2/CH_3CN 2:1) and subsequent concentration afforded **9** (735 mg, 84%) as a light yellow oil. ^{1}H NMR ($CDCl_3$): $\delta=3.58$ (t, $J=6.2$ Hz, 2H), 2.71 (m, 2H), 2.58 (s, 3H), 1.95–1.81 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta=$ 129.8, 52.9, 44.3, 38.3, 28.5, 19.6 ppm; HRMS (ESI): *m/z*: calcd for $[M+Na]^+$: 200.0180; found: 200.0172.

1-Isothiocyanato-4-(methylsulfonyl)butane (10): A solution of *m*-CPBA (964 mg, 5.59 mmol) in anhydrous CH_2Cl_2 (5.0 mL) was slowly added to a solution of **8** (283 mg, 1.75 mmol) in anhydrous CH_2Cl_2 (2.5 mL). After 2 h of stirring at ambient temperature, the mixture was diluted with CH_2Cl_2 (100 mL) and the organics were washed with satd. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. Silica gel chromatography (CH_2Cl_2) and subsequent concentration afforded **10** (203 mg, 60%) as an off-white solid. ^{1}H NMR ($CDCl_3$): $\delta=3.56$ (t, $J=6.1$ Hz, 2H), 3.01 (t, $J=7.8$ Hz, 2H), 2.86 (s, 3H), 1.90 (m, 2H), 1.81 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): $\delta=$ 130.4, 53.5, 44.4, 40.6, 28.4, 19.6 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 193.0231; found: 193.0230.

Method A: **Isothiocyanate installation with thiophosgene.** A solution of thiophosgene (0.50 M, 3 equiv) in anhydrous CH_2Cl_2 was chilled to 0 °C. A solution of the primary amine in anhydrous CH_2Cl_2 (1 mL mmol^{-1}) was added. If the hydrochloride salt of the amine was used, it was first neutralized with diisopropylethylamine (DIEA, 1–2 equiv). Finely crushed NaOH (3 equiv) was then added and the resulting solution was allowed to warm to ambient temperature over 3 h. Products were concentrated in vacuo and any resulting solids were removed by filtration through celite.

Method B: **Isothiocyanate installation with di(pyridin-2-yl) thionocarbonate**: The primary amine was dissolved in anhydrous CH_2Cl_2 (14.5 mL mmol^{-1}) at ambient temperature, and di(pyridin-2-yl) thionocarbonate (1 equiv) was added. The reaction mixture was stirred for 24 h, followed by solvent removal in vacuo. (General Method B is the preferred method for conversion of a primary amine into an isothiocyanate for reasons of safety, general utility, and ease of use. Although only one isothiocyanate in this report was constructed by this method, our laboratory has readily employed this method for the construction of several as-of-yet unreported isothiocyanates.)

1-Isothiocyanato-2-methylpropane (11): Compound **11** was synthesized by Method A from thiophosgene (206 μL, 311 mg, 2.71 mmol), isobutylamine (96 μL, 70 mg, 0.96 mmol), and NaOH (133 mg, 3.32 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **11** (31 mg, 28%) as an orange oil. ^{1}H NMR ($CDCl_3$): $\delta=3.34$ (d, $J=6.2$ Hz, 2H), 2.00 (nonet, $J=6.7$ Hz, 1H), 1.01 ppm (d, $J=6.7$ Hz, 6H); ^{13}C NMR ($CDCl_3$): $\delta=$ 129.8, 52.6, 29.8, 19.9 ppm.

1-Isothiocyanato-2,2-dimethylpropane (12): Compound **12** was synthesized by Method A from thiophosgene (196 μL, 296 mg, 2.57 mmol), neopentylamine (112 μL, 83 mg, 0.85 mmol), and NaOH (137 mg, 3.42 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **12** (55 mg, 40%) as a light orange oil. ^{1}H NMR ($CDCl_3$): $\delta=3.26$ (s, 2H), 1.02 ppm (s, 9H); ^{13}C NMR ($CDCl_3$): $\delta=57.3$, 33.5, 27.1 ppm.

Isothiocyanatocyclopropane (13): Compound **13** was synthesized by Method A from thiophosgene (903 μL, 1.36 g, 11.85 mmol), cyclopropylamine (271 μL, 221 mg, 3.87 mmol), and NaOH (488 mg, 12.20 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **13** (35 mg, 9%) as an orange oil. ^{1}H NMR ($CDCl_3$): $\delta=2.89$ (tt, $J=7.0$, 3.8 Hz, 1H), 0.93–0.87 (m, 2H), 0.87–0.81 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): $\delta=126.7$, 25.5, 8.3 ppm.

(Isothiocyanatomethyl)cyclohexane (14): Compound **14** was synthesized by Method A from thiophosgene (206 μL, 311 mg, 2.71 mmol), cyclohexylmethylamine (125 μL, 109 mg, 0.96 mmol), and NaOH (131 mg, 3.27 mmol). Silica gel chromatography (hexane/CH_2Cl_2 3:1) and subsequent concentration afforded **14** (138 mg, 92%) as an orange oil. ^{1}H NMR ($CDCl_3$): $\delta=3.33$ (d, $J=$ 6.3 Hz, 2H), 1.80–1.71 (m, 4H), 1.17–1.59 (m, 2H), 1.32–1.07 (m, 3H), 1.06–0.94 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): $\delta=129.5$, 51.3, 38.7, 30.4, 26.1, 25.7 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 155.0769; found: 155.0771.

1-Isothiocyanatobenzene (15): Compound **15** was synthesized by Method A from thiophosgene (182 μL, 274 mg, 2.38 mmol), aniline (87 μL, 89 mg, 0.96 mmol), and NaOH (127 mg, 3.17 mmol). Silica gel chromatography (hexane/CH_2Cl_2 25:1) and subsequent concentration afforded **15** as a colorless oil in quantitative yield. ^{1}H NMR ($CDCl_3$): $\delta=7.33$ (m, 2H), 7.26 (m, 1H), 7.20 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): $\delta=135.5$, 131.4, 129.7, 127.5, 125.9 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 135.0143; found: 135.0149.

1-Bromo-4-isothiocyanatobenzene (16): Compound **16** was synthesized by Method A from thiophosgene (208 μL, 314 mg, 2.73 mmol), 4-bromoaniline (163 mg, 0.95 mmol), and NaOH (144 mg, 3.60 mmol). Silica gel chromatography (hexane/CH_2Cl_2 25:1) and subsequent concentration afforded **16** (168 mg, 83%) as a white solid. ^{1}H NMR ($CDCl_3$): $\delta=7.46$ (d, $J=8.7$ Hz, 2H), 7.08 ppm

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(d, J=8.7 Hz, 2H); ^{13}C NMR (CDCl$_3$): δ=137.1, 132.9, 130.7, 127.3, 120.9 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 212.9248; found: 212.9245.

1-Butyl-4-isothiocyanatobenzene (17): Compound **17** was synthesized by Method A from thiophosgene (208 μL, 314 mg, 2.73 mmol), 4-butylaniline (150 μL, 140 mg, 0.94 mmol), and NaOH (123 mg, 3.07 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 25:1) and subsequent concentration afforded **17** as a light yellow oil in quantitative yield. ^{1}H NMR (CDCl$_3$): δ=7.16 (d, J=8.6 Hz, 2H), 7.14 (d, J=8.6 Hz, 2H), 2.62 (t, J=7.8 Hz, 2H), 1.60 (m, 2H), 1.37 (sextet, J=7.4 Hz, 2H), 0.95 ppm (t, J=7.4 Hz, 3H); ^{13}C NMR (CDCl$_3$): δ=142.7, 134.8, 129.6, 128.7, 125.7, 35.4, 33.5, 22.4, 14.0 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 191.0769; found: 191.0777.

3-(Isothiocyanatomethyl)pyridine (18): Compound **18** was synthesized by Method B from 3-(isothiocyanatomethyl)pyridine (140 μL, 150 mg, 1.39 mmol) and di(pyridin-2-yl)thionocarbonate (325 mg, 1.40 mmol). Silica gel chromatography (hexane/EtOAc 1:2) and subsequent concentration afforded **18** (190 mg, 91%) as a yellow oil. ^{1}H NMR (CDCl$_3$): δ=8.62 (d, J=4.4 Hz, 1H), 8.59 (s, 1H), 7.70 (dt, J=7.9, 1.718 Hz, 1H), 7.36 (dd, J=7.8, 4.8 Hz, 1H), 4.76 ppm (s, 2H); ^{13}C NMR (CDCl$_3$): δ=149.7, 148.2, 134.6, 133.7, 130.1, 123.7, 46.4 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 150.0252; found: 150.0248.

1-(Isothiocyanatomethyl)benzene (19): Compound **19** was synthesized by Method A from thiophosgene (480 μL, 724 mg, 6.30 mmol), benzylamine (200 μL, 196 mg, 1.83 mmol), and NaOH (224 mg, 5.61 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 5:1) and subsequent concentration afforded **19** (127 mg, 46%) as a orange oil. ^{1}H NMR (CDCl$_3$): δ=7.34 (m, 5H), 4.68 ppm (s, 2H); ^{13}C NMR (CDCl$_3$): δ=134.1, 131.9, 128.8, 128.2, 126.7, 48.5 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 149.0299; found: 149.0303.

2-(Isothiocyanatomethyl)furan (20): Compound **20** was synthesized by Method A from thiophosgene (206 μL, 311 mg, 2.71 mmol), furfurylamine (89 μL, 93 mg, 0.96 mmol), and NaOH (125 mg, 3.12 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 3:1) and subsequent concentration afforded **20** (37 mg, 27%) as an orange oil. ^{1}H NMR (CDCl$_3$): δ=7.43 (m, 1H), 6.37 (dd, J=3.2, 1.8 Hz, 1H), 6.35 (bd, J=3.2 Hz, 1H), 4.66 ppm (s, 2H); ^{13}C NMR (CDCl$_3$): δ=147.5, 143.4, 135.1, 110.8, 108.9, 42.1 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 139.0092; found: 139.0092.

1-Bromo-2-(isothiocyanatomethyl)benzene (21): Compound **21** was synthesized by Method A from thiophosgene (208 μL, 314 mg, 2.731 mmol), 2-bromobenzylamine hydrochloride (213 mg, 0.96 mmol), DIEA (250 μL, 186 mg, 1.44 mmol), and NaOH (155 mg, 3.87 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 5:1) and subsequent concentration afforded **21** (209 mg, 95%) as a reddish-orange oil. ^{1}H NMR (CDCl$_3$): δ=7.59 (dd, J=8.0, 0.8 Hz, 1H), 7.46 (dd, J=7.7, 1.2 Hz, 1H), 7.38 (td, J=7.4, 0.9 Hz, 1H), 7.23 (td, J=7.7, 1.5 Hz, 1H), 4.81 ppm (s, 2H); ^{13}C NMR (CDCl$_3$): δ=133.8, 133.4, 133.1, 130.1, 128.9, 128.1, 122.5, 49.3 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 226.9404; found: 226.9405.

1-Bromo-4-(isothiocyanatomethyl)benzene (22): Compound **22** was synthesized by Method A from thiophosgene (196 μL, 296 mg, 2.58 mmol), 4-bromobenzylamine (184 mg, 0.99 mmol), and NaOH (123 mg, 3.07 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 5:1) and subsequent concentration afforded **22** (193 mg, 85%) as a reddish-orange oil. ^{1}H NMR (CDCl$_3$): δ=7.51 (d, J=8.4 Hz, 2H), 7.19 (d, J=8.4 Hz, 2H), 4.67 ppm (s, 2H); ^{13}C NMR (CDCl$_3$): δ=133.4, 133.3, 132.2, 128.7, 122.5, 48.3 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 226.9404; found: 226.9410.

2-(Isothiocyanatomethyl)-1,2-dimethoxybenzene (23): Compound **23** was synthesized by Method A from thiophosgene (182 μL, 274 mg, 2.39 mmol), 2,3-dimethoxybenzylamine (167 mg, 1.00 mmol), and NaOH (130 mg, 3.25 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 4:1 to hexane/CH$_2$Cl$_2$ 3:1) and subsequent concentration afforded **23** (164 mg, 78%) as a light yellow oil. ^{1}H NMR (CDCl$_3$): δ=7.08 (t, J=8.0 Hz, 1H), 6.93 (m, 2H), 4.72 (s, 2H), 3.89 (s, 3H), 3.87 ppm (s, 3H); ^{13}C NMR (CDCl$_3$): δ=152.7, 146.6, 131.5, 128.0, 124.4, 120.3, 113.0, 61.0, 55.9, 44.1 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 209.0511; found: 209.0502.

2-(Isothiocyanatomethyl)-1,3,5-trimethoxybenzene (24): Compound **24** was synthesized by Method A from thiophosgene (182 μL, 274 mg, 2.39 mmol), 2,4,6-trimethyoxybenzylamine (185 mg, 0.94 mmol), and NaOH (123 mg, 3.07 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 1:1 to CH$_2$Cl$_2$) and subsequent concentration afforded **24** (218 mg, 97%) as a light yellow oil. ^{1}H NMR (CDCl$_3$): δ=6.52 (s, 2H), 4.64 (s, 2H), 3.87 (s, 6H), 3.84 ppm (s, 3H); ^{13}C NMR (CDCl$_3$): δ=153.8, 138.1, 133.3, 130.1, 104.1, 61.0, 56.4, 49.1 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 239.0616; found: 239.0626.

5-(Isothiocyanatomethyl)benzo[d][1,3]dioxole (25): Compound **25** was synthesized by Method A from thiophosgene (220 μL, 332 mg, 2.89 mmol), 3,4-methylenedioxybenzylamine (144 mg, 0.96 mmol), and NaOH (120 mg, 3.00 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 5:1) and subsequent concentration afforded **25** (84 mg, 45%) as a off-white solid. ^{1}H NMR (CDCl$_3$): δ=6.79 (m, 3H), 5.98 (s, 2H), 4.60 ppm (s, 2H); ^{13}C NMR (CDCl$_3$): δ=148.3, 147.9, 132.5, 128.1, 120.8, 108.6, 107.8, 101.6, 48.8 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 193.0198; found: 193.0202.

1-(Isothiocyanatomethyl)naphthalene (26): Compound **26** was synthesized by Method A from thiophosgene (220 μL, 332 mg, 2.89 mmol), 1-(methylamine)naphthalene (140 μL, 150 mg, 0.95 mmol), and NaOH (122 mg, 3.06 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 5:1) and subsequent concentration afforded **26** (137 mg, 72%) as an off-white solid. ^{1}H NMR (CDCl$_3$): δ=7.86 (m, 3H), 7.60–7.41 (m, 4H), 5.07 ppm (s, 2H); ^{13}C NMR (CDCl$_3$): δ=133.9, 133.0, 130.6, 129.9, 129.6, 129.2, 127.2, 126.4, 126.0, 125.5, 122.7, 47.3 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 199.0456; found: 199.0462.

(*S*)-1-Isothiocyanato-1,2,3,4-tetrahydronaphthalene (27): Compound **27** was synthesized by Method A from thiophosgene (208 μL, 314 mg, 2.73 mmol), (*S*)-1,2,3,4-tetrahydronaphthaleneamine (138 μL, 142 mg, 0.97 mmol), and NaOH (137 mg, 3.42 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 5:1) and subsequent concentration afforded **27** (159 mg, 87%) as a yellow-orange oil. ^{1}H NMR (CDCl$_3$): δ=7.33 (m, 1H), 7.21 (m, 2H), 7.10 (m, 1H), 4.90 (t, J=5.3 Hz, 1H), 2.83 (dt, J=17.0, 5.9 Hz, 1H), 2.72 (ddd, J=17.0, 7.75, 6.1 Hz, 1H), 2.08 (m, 2H), 1.96 (m, 1H), 1.81 ppm (m, 1H); ^{13}C NMR (CDCl$_3$): δ=136.5, 133.3, 132.0, 129.6, 128.7, 128.4, 126.6, 55.9, 30.9, 28.7, 19.4 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 189.0612; found: 189.0610.

(*R*)-1-Isothiocyanato-1,2,3,4-tetrahydronaphthalene (28): Compound **28** was synthesized by Method A from thiophosgene (182 μL, 274 mg, 2.39 mmol), (*R*)-1,2,3,4-tetrahydronaphthaleneamine (138 μL, 142 mg, 0.97 mmol), and NaOH (125 mg, 3.12 mmol). Silica gel chromatography (hexane/CH$_2$Cl$_2$ 5:1) and subsequent concentration afforded **28** (183 mg, 100%) as a light yellow oil. ^{1}H NMR (CDCl$_3$): δ=7.32 (m, 1H), 7.20 (m, 2H), 7.10 (m, 1H), 4.89

(t, $J=5.4$ Hz, 1 H), 2.83 (dt, $J=17.0$, 6.0 Hz, 1 H), 2.72 (ddd, $J=17.0$, 7.6, 6.0 Hz, 1 H), 2.08 (m, 2 H), 1.96 (m, 1 H), 1.81 ppm (m, 1 H); ^{13}C NMR ($CDCl_3$): $\delta=136.5$, 133.3, 132.0, 129.5, 128.6, 128.4, 126.6, 55.8, 30.9, 28.7, 19.4 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 189.0612; found: 189.0603.

1-(Isothiocyanatomethyl)-2-phenylbenzene (29): Compound **29** was synthesized by Method A from thiophosgene (196 μL, 296 mg, 2.57 mmol), (biphenyl-2-yl)methyl amine hydrochloride (213 mg, 0.97 mmol), DIEA (250 μL, 186 mg, 1.44 mmol), and NaOH (134 mg, 3.35 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **29** (189 mg, 87%) as a deep orange oil. ^{1}H NMR ($CDCl_3$): $\delta=7.49$–7.45 (m, 1 H), 7.43–7.32 (m, 5 H), 7.27–7.23 (m, 3 H), 4.55 ppm (s, 2 H); ^{13}C NMR ($CDCl_3$): $\delta=$ 141.4, 139.8, 132.0, 131.8, 130.4, 129.09, 128.6, 128.6, 128.4, 128.2, 127.8, 47.1 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 225.0612; found: 225.0618.

1-(Isothiocyanatomethyl)-3-phenylbenzene (30): Compound **30** was synthesized by Method A from thiophosgene (196 μL, 296 mg, 2.57 mmol), (biphenyl-2-yl)methyl amine hydrochloride (220 mg, 1.00 mmol), DIEA (250 μL, 186 mg, 1.44 mmol), and NaOH (134 mg, 3.35 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **30** as a deep orange oil in quantitative yield. ^{1}H NMR ($CDCl_3$): $\delta=7.56$–7.48 (m, 3 H), 7.47–7.37 (m, 4 H), 7.33 (m, 1 H), 7.22 (m, 1 H), 4.66 ppm (s, 2 H); ^{13}C NMR ($CDCl_3$): $\delta=142.1$, 140.4, 134.9, 132.6, 129.5, 129.0, 127.8, 127.2, 127.2, 125.7, 125.6, 48.8 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 225.0612; found: 225.0609.

1-(Isothiocyanatomethyl)-4-phenylbenzene (31): Compound **31** was synthesized by Method A from thiophosgene (220 μL, 332 mg, 2.89 mmol), (biphenyl-4-yl)methyl amine (177 mg, 0.97 mmol), and NaOH (122 mg, 3.07 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **31** (137 mg, 64%) as a off-white solid. ^{1}H NMR ($CDCl_3$): $\delta=7.58$ (m, 4 H), 7.43 (m, 2 H), 7.35 (m, 3 H), 4.71 ppm (s, 2 H); ^{13}C NMR ($CDCl_3$): $\delta=141.5$, 140.5, 133.3, 132.6, 129.0, 127.8, 127.8, 127.5, 127.2, 48.6 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 225.0612; found: 225.0622.

1-(Isothiocyanatomethyl)-4-phenoxybenzene (32): Compound **32** was synthesized by Method A from thiophosgene (196 μL, 296 mg, 2.57 mmol), 4-phenoxybenzylamine (198 mg, 0.99 mmol), and NaOH (125 mg, 3.12 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **32** (104 mg, 43%) as a light orange oil. ^{1}H NMR ($CDCl_3$): $\delta=7.34$ (m, 2 H), 7.25 (m, 2 H), 7.12 (tt, $J=7.4$, 1.1 Hz, 1 H), 7.02–6.98 (m, 4 H), 4.65 ppm (s, 2 H); ^{13}C NMR ($CDCl_3$): $\delta=157.7$, 156.9, 132.5, 130.0, 129.0, 128.7, 123.9, 119.3, 119.1, 48.4 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 241.0561; found: 241.0550.

4-Isothiocyanato-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (33): Compound **33** was synthesized by Method A from thiophosgene (208 μL, 314 mg, 2.73 mmol), 4-aminoantipyrine (196 mg, 0.96 mmol), and NaOH (110 mg, 2.75 mmol). Silica gel chromatography (EtOAc) and subsequent concentration afforded **33** (235 mg, 99%) as a light yellow solid. ^{1}H NMR ($CDCl_3$): $\delta=7.45$ (t, $J=7.7$ Hz, 2 H), 7.32 (m, 3 H), 3.10 (s, 3 H), 2.27 ppm (s, 3 H); ^{13}C NMR ($CDCl_3$): $\delta=160.9$, 147.9, 142.9, 134.1, 129.3, 127.5, 124.5, 103.6, 35.7, 10.9 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 245.0623; found: 245.0635).

1-(2-Isothiocyanatoethyl)benzene (34): Compound **34** was synthesized by Method A from thiophosgene (196 μL, 296 mg, 2.57 mmol), 2-phenylethylamine (122 μL, 117 mg, 0.97 mmol), and NaOH (131 mg, 3.27 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **34** (97 mg, 61%) as a light orange oil. ^{1}H NMR ($CDCl_3$): $\delta=7.33$ (m, 2 H), 7.26 (m, 1 H), 7.21 (m, 2 H), 3.70 (t, $J=7.0$ Hz, 2 H), 2.97 ppm (t, $J=7.0$ Hz, 2 H); ^{13}C NMR ($CDCl_3$): $\delta=137.1$, 131.0, 128.9, 127.3, 46.5, 36.6 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 163.0456; found: 163.0463.

1-(2-Isothiocyanatoethyl)cyclohex-1-ene (35): Compound **35** was synthesized by Method A from thiophosgene (206 μL, 311 mg, 2.71 mmol), 2-(cyclohex-1-enyl)ethylamine (135 μL, 121 mg, 0.97 mmol), and NaOH (140 mg, 3.50 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **35** as an orange oil in quantitative yield. ^{1}H NMR ($CDCl_3$): $\delta=5.54$ (m, 1 H), 3.53 (t, $J=6.7$ Hz, 2 H), 2.31 (t, $J=6.8$ Hz, 2 H), 2.01 (m, 2 H), 1.91 (m, 2 H), 1.67–1.60 (m, 2 H), 1.60–1.52 ppm (m, 2 H); ^{13}C NMR ($CDCl_3$): $\delta=132.8$, 131.0, 125.6, 43.8, 38.7, 27.9, 25.4, 22.9, 22.3 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 163.0769; found: 163.0771.

2-Isothiocyanato-1,1-diphenylethane (36): Compound **36** was synthesized by Method A from thiophosgene (220 μL, 332 mg, 2.89 mmol), 2,2-diphenylethylamine (192 mg, 0.97 mmol), and NaOH (123 mg, 3.06 mmol). Silica gel chromatography (hexane/CH_2Cl_2 5:1) and subsequent concentration afforded **36** as a light yellow oil in quantitative yield. ^{1}H NMR ($CDCl_3$): $\delta=7.30$ (m, 4 H), 7.25–7.16 (m, 6 H), 4.31 (t, $J=7.6$, 1 H), 3.99 ppm (d, $J=7.5$ Hz, 2 H); ^{13}C NMR ($CDCl_3$): $\delta=140.4$, 131.9, 128.9, 128.0, 127.4, 51.4, 49.4 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 239.0769; found: 239.0763.

3,3-Diphenylpropanal (51): 3,3-Diphenylpropanol (1.00 g, 4.72 mmol) was dissolved in anhydrous CH_2Cl_2 (45 mL), and this solution was stirred at ambient temperature for 15 min. Dess–Martin periodinane in CH_2Cl_2 (15% *w/v*, 16.5 mL, 9.10 mmol) was added to this solution. The mixture was stirred for 2 h until TLC indicated nearly full consumption of the starting alcohol. The mixture was diluted with Et_2O (150 mL) and stirred with satd. Na_2SO_3 (75 mL) and satd. $NaHCO_3$ (75 mL) for 10 min until the organic phase became clear. The organics were washed with satd. $NaHCO_3$ and brine and were dried over Na_2SO_4 prior to concentration in vacuo. Silica gel chromatography (hexane/CH_2Cl_2 1:1) and subsequent concentration afforded **51** (868 mg, 88%) as a colorless oil. ^{1}H NMR ($CDCl_3$): $\delta=9.67$ (t, $J=1.8$ Hz, 1 H), 7.29–7.14 (m, 10 H), 4.59 (t, $J=7.7$ Hz, 1 H), 3.12 ppm (dd, $J=7.7$, 1.8 Hz, 2 H); ^{13}C NMR ($CDCl_3$): $\delta=201.1$, 143.4, 128.8, 127.9, 126.8, 49.5, 45.1 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 210.1045; found: 210.1041.

3,3-Diphenylpropanal oxime (56): Compound **51** (151 mg, 0.72 mmol) was dissolved in EtOH (95%, 2.7 mL) and pyridine (268 μL, 262 mg, 3.31 mmol), and hydroxylamine hydrochloride (94 mg, 1.35 mmol) was added. This solution was heated to reflux for 2.5 h, followed by concentration in vacuo. Products were dissolved in H_2O/EtOAc (1:1 100 mL), and the aqueous phase was extracted with EtOAc (3×50 mL). Combined organics were washed with brine and dried over Na_2SO_4 prior to concentration in vacuo to afford **56** (152 mg, 94%) as an off-white solid. Ratio of isomers: 1.77:1. ^{1}H NMR ($CDCl_3$): $\delta=9.65$ (br s, 1 H), 9.16 (br s, 1 H), 7.29 (t, $J=6.1$ Hz, 1 H), 7.27–7.12 (m, 10 H), 6.63 (t, $J=5.1$ Hz, 1 H), 4.18 (t, $J=8.3$ Hz, 1 H), 4.13 (t, $J=8.1$ Hz, 1 H), 3.11 (dd, $J=8.2$, 5.1 Hz, 2 H), 2.92 ppm (dd, $J=8.1$, 6.2 Hz, 2 H); ^{13}C NMR ($CDCl_3$): $\delta=150.9$, 150.8, 143.7, 143.5, 128.8, 128.0, 127.9, 126.7, 49.2, 48.0, 35.5, 31.6 ppm; HRMS (ESI): *m/z*: calcd for $[M+H]^+$: 226.1232; found: 226.1235.

(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1-(2,2-diphenylethyl)thiohydroximate (58): Compound **56** (202 mg, 0.90 mmol) was dissolved in DMF (5.9 mL), and *N*-chlorosuccinimide (123 mg, 0.92 mmol) was slowly added in portions. The solution was heated to 50–70 °C for 2 h and then cooled to ambient temperature. 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucose (321 mg, 0.88 mmol) in DMF (5.85 mL) and anhydrous diisopropylethylamine (1.39 mL, 1.04 g, 8.02 mmol) were added to this solution. The resulting solution was stirred at ambient temperature for 18 h. The mixture was diluted with Et_2O and washed with H_2SO_4 (1 M). The remaining aqueous phase was extracted with EtOAc (3×50 mL). The organics were combined, dried over $MgSO_4$, and concentrated in vacuo. Residual DMF was removed by co-stripping with Et_2O and mild heating in vacuo. Silica gel chromatography (hexane/CH_2Cl_2/MeOH 6:3:1), concentration in vacuo, and subsequent lyophilization from H_2O/CH_3CN afforded **58** (517 mg, 100 %) as a white solid. ^{1}H NMR ($CDCl_3$): $\delta = 8.78$ (br s, 1 H), 7.33–7.14 (m, 10 H), 5.17 (t, $J = 9.4$ Hz, 1 H), 5.05 (t, $J = 9.9$ Hz, 1 H), 5.02 (t, $J = 9.4$ Hz, 1 H), 4.92 (d, $J = 10.3$ Hz, 1 H), 4.51 (AB**X**, $J_{AX} = 7.6$ Hz, $J_{BX} = 7.4$ Hz, 1 H), 4.14 (**A**BM, $J_{AB} = 12.4$ Hz, $J_{AM} = 5.8$ Hz, 1 H), 4.05 (A**B**M, $J_{AB} = 12.4$ Hz, $J_{BM} = 1.6$ Hz, 1 H), 3.63 (AB**M**X, $J_{MX} = 9.8$ Hz, $J_{BM} = 5.8$ Hz, $J_{AM} = 1.6$ Hz, 1 H), 3.31 (**A**BX, $J_{AB} = 15.4$ Hz, $J_{AX} = 7.6$ Hz, 1 H), 3.15 (A**B**X, $J_{AB} = 15.4$ Hz, $J_{BX} = 7.4$ Hz, 1 H), 2.02 (s, 3 H), 2.00 (s, 3 H), 1.98 (s, 3 H), 1.91 ppm (s, 3 H); ^{13}C NMR ($CDCl_3$): $\delta = 170.9$, 170.4, 169.5, 169.4, 149.9, 143.9, 143.2, 128.8, 128.7, 128.0, 127.8, 126.8, 126.7, 80.1, 76.0, 73.8, 70.2, 68.1, 62.4, 48.4, 38.8, 20.7, 20.7, 20.6 ppm; HRMS (ESI): *m/z*: calcd for $[M+Na]^+$: 610.1723; found: 610.1743.

(2,3,4,6-Tetra-*O*-acetyl)-1-(2,2-diphenylethyl)glucosinolate (60): Anhydrous pyridine (7.80 mL, 7.63 g, 96.44 mmol) was dissolved in CH_2Cl_2 (8.5 mL), and the system was cooled to 0 °C. A solution of chlorosulfonic acid (700 μL, 1.22 g, 10.49 mmol) in CH_2Cl_2 (8.5 mL) was slowly added over 5 min. The resulting solution was warmed to ambient temperature, a solution of **58** (298 mg, 0.51 mmol) in CH_2Cl_2 (5.9 mL) was added, and the solution was stirred at ambient temperature for 18 h. A solution of $NaHCO_3$ (1.29 g, 15.38 mmol) in H_2O (13.5 mL) was slowly added, and solvents were concentrated in vacuo. The remaining solution was extracted with EtOAc (3×50 mL) and the combined organics were dried over Na_2SO_4 and concentrated in vacuo. Two-step silica gel chromatography (EtOAc/hexane/MeOH 6:3:1 to EtOAc/MeOH 4:1, $R_f = 0.05$ in EtOAc/hexane/MeOH 6:3:1), concentration in vacuo, and subsequent lyophilization from H_2O/CH_3CN afforded **60** (275 mg, 79 %) as a white solid. ^{1}H NMR (CD_3OD): $\delta = 7.38$–7.33 (m, 4 H), 7.31–7.25 (m, 4 H), 7.21–7.14 (m, 2 H), 5.27 (t, $J = 9.7$ Hz, 1 H), 5.08 (d, $J = 10.1$ Hz, 1 H), 5.01 (t, $J = 9.8$ Hz, 1 H), 4.94 (dd, $J = 9.9$, 9.3 Hz, 1 H), 4.71 (AB**X**, $J_{BX} = 8.7$ Hz, $J_{AX} = 6.7$ Hz, 1 H), 4.17 (**A**BX, $J_{AB} = 12.4$ Hz, $J_{AX} = 6.3$ Hz, 1 H), 4.11 (A**B**X, $J_{AB} = 12.4$ Hz, $J_{BX} = 1.7$ Hz, 1 H), 3.94 (AB**X**Y, $J_{XY} = 10.0$ Hz, $J_{AX} = 6.3$ Hz, $J_{BX} = 1.7$ Hz, 1 H), 3.45 (**A**BX, $J_{AB} = 15.4$ Hz, $J_{AX} = 6.7$ Hz, 1 H), 3.38 (A**B**X, $J_{AB} = 15.4$ Hz, $J_{BX} = 8.7$ Hz, 1 H), 2.01 (s, 3 H), 1.99 (s, 3 H), 1.96 (s, 3 H), 1.86 ppm (s, 3 H); ^{13}C NMR (CD_3OD): $\delta = 172.4$, 171.5, 171.3, 171.0, 157.8, 145.3, 129.7, 129.6, 129.5, 129.1, 127.8, 127.6, 80.9, 76.9, 74.9, 71.1, 69.6, 63.8, 49.7, 39.7, 20.7, 20.7, 20.7, 20.6 ppm; HRMS (ESI): *m/z*: calcd for $[M+Na]^+$: 712.1110; found: 712.1098.

1-(2,2-Diphenylethyl)glucosinolate (38): Compound **60** (94.7 mg, 0.137 mmol) was dissolved in anhydrous MeOH (1.9 mL), and NaOMe in MeOH (0.5 M, 145 μL, 0.073 mmol) was added. The solution was stirred at ambient temperature for 1 h, after which AcOH (500 μL) was added. Concentration in vacuo, followed by lyophilization from H_2O/CH_3CN 40:1, afforded **38** as a white solid in quantitative yield. ^{1}H NMR (CD_3OD): $\delta = 7.31$–7.24 (m, 4 H), 7.22–7.16 (m, 4 H), 7.11–7.04 (m, 2 H), 4.63 (AB**X**, $J_{AX} = 7.6$ Hz, $J_{BX} = 7.2$ Hz, 1 H), 4.62 (d, $J = 9.5$ Hz, 1 H), 3.75 (**A**BX, $J_{AB} = 12.2$ Hz, $J_{AX} = 2.0$ Hz, 1 H), 3.56 (A**B**X, $J_{AB} = 12.2$ Hz, $J_{BX} = 5.8$ Hz, 1 H), 3.44 (**A**BX, $J_{AB} = 15.4$ Hz, $J_{AX} = 7.6$ Hz, 1 H), 3.33 (A**B**X, $J_{AB} = 15.4$ Hz, $J_{BX} = 7.2$ Hz, 1 H), 3.28–3.22 (m, 2 H), 3.20–3.13 ppm (m, 2 H); ^{13}C NMR (CD_3OD): $\delta = 160.0$, 145.9, 145.1, 129.6, 129.5, 129.3, 129.3, 127.6, 127.4, 83.9, 82.4, 79.5, 74.3, 71.2, 62.7, 49.6, 40.0 ppm; HRMS (ESI): *m/z*: calcd for $[M+Na]^+$: 544.0688; found: 544.0687.

(2-Biphenyl-2-ylmethyl)-1,3-dithiane (53): The 1,3-dithiane (1.91 g, 15.86 mmol) was dissolved in anhydrous THF (14 mL), and the system was cooled to −40 °C (CH_3CN/CO_2). A solution of *n*BuLi in hexanes (10 M, 2.00 mL, 20.00 mmol) was added, and the reaction was allowed to proceed at −40 °C for 30 min, after which the mixture was warmed to −20 °C (CCl_4/CO_2) for 1.5 h. The solution was then re-cooled to −40 °C, (biphenyl-2-yl)methyl bromide (2.96 mL, 16.21 mmol) was added, and the mixture was stirred at −40 °C for 1.5 h. Excess *n*BuLi was quenched with water (20 mL), and the solution was extracted with CH_2Cl_2 (3×60 mL). Combined organics were washed with satd. $NaHCO_3$ and brine and were dried over Na_2SO_4 prior to concentration in vacuo. Silica gel chromatography (hexane/CH_2Cl_2 5:1, $R_f = 0.13$) and subsequent concentration afforded **53** (2.95 g, 65 %) as a light green oil. ^{1}H NMR ($CDCl_3$): $\delta = 7.43$ (m, 9 H), 3.97 (t, $J = 7.6$ Hz, 1 H), 3.08 (d, $J = 7.6$ Hz, 2 H), 2.70–2.61 (m, 4 H), 2.02–1.94 (m, 1 H), 1.82–1.65 ppm (m, 1 H); ^{13}C NMR ($CDCl_3$): $\delta = 142.5$, 141.4, 134.9, 130.4, 130.1, 129.4, 128.4, 127.4, 127.2, 127.0, 48.0, 38.7, 30.4, 25.8 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 286.0850; found: 286.0844.

2-(Biphenyl-2-yl)ethanal (54): Compound **53** (2.95 g, 10.31 mmol) was dissolved in CH_3CN/CH_2Cl_2/H_2O (8:1:1, 51.5 mL), a solution of Dess–Martin periodinane (15 % *w/v*, 38.20 mL, 21.06 mmol) was added, and the mixture was stirred at ambient temperature for 18 h. Satd. Na_2SO_3 (100 mL) was added, and the mixture was extracted with Et_2O (3×200 mL), followed by EtOAc (1×200 mL). The organics were separated and washed with satd. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. Silica gel chromatography (hexane/CH_2Cl_2 1:1, $R_f = 0.35$) and subsequent concentration afforded **54** (918 mg, 45 %) as a colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 9.62$ (t, $J = 2.0$ Hz, 1 H), 7.48–7.23 (m, 9 H), 3.68 ppm (d, $J = 2.1$ Hz, 2 H); ^{13}C NMR ($CDCl_3$): $\delta = 199.8$, 143.1, 141.0, 130.8, 130.6, 130.1, 129.3, 128.6, 128.0, 127.7, 127.6, 48.5 ppm; HRMS (EI): *m/z*: calcd for $[M]^+$: 196.0888; found: 196.0887.

2-(Biphenyl-2-yl)ethanal oxime (57): Compound **54** (918 mg, 4.68 mmol) was dissolved in EtOH (95 %, 17.55 mL) and pyridine (1.75 mL, 1.71 g, 21.64 mmol), and hydroxylamine hydrochloride (636 mg, 9.15 mmol) was added. This solution was heated to reflux for 2.5 h, followed by concentration in vacuo. Products were dissolved in H_2O/EtOAc (1:1, 100 mL), and the aqueous phase was extracted with EtOAc (3×50 mL). Combined organics were dried over Na_2SO_4 and co-stripped with EtOAc (3×25 mL) in vacuo to afford **57** (992 mg, 100 %) as an off-white solid. Ratio of isomers: 1.16:1. ^{1}H NMR ($CDCl_3$): $\delta = 8.42$ (br s, 1 H), 8.08 (br s, 1 H), 7.44–7.24 (m, 10 H), 6.73 (t, $J = 5.2$ Hz, 1 H), 3.71 (d, $J = 5.2$ Hz, 2 H), 3.49 ppm (d, 6.1 Hz, 2 H); ^{13}C NMR ($CDCl_3$): $\delta = 151.0$, 142.5, 142.4, 141.2, 134.4, 133.8, 130.5, 129.0, 129.4, 129.4, 128.5, 128.5, 128.0, 12.9, 127.4, 127.3, 127.1, 127.0, 33.7, 29.8 ppm; HRMS (ESI): *m/z*: calcd for $[M+H]^+$: 212.1075; found: 212.1068.

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-1-(biphenyl-2-yl)methyl thiohydroximate (59): Compound **57** (203 mg, 0.96 mmol) was dissolved in DMF (6.15 mL), and *N*-chlorosuccinimide (129 mg, 0.97 mmol) was slowly added in portions. The solution was heated to 50–70 °C for 2 h and then cooled to ambient temperature. 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucose (338 mg, 0.93 mmol) in DMF (6.15 mL) was added to this solution, followed by anhydrous diisopropylethylamine (1.50 mL, 1.12 g, 8.65 mmol). The resulting solution was stirred at ambient temperature for 18 h. The mixture

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was diluted with Et_2O and washed with H_2SO_4 (1 M). The remaining aqueous phase was extracted with EtOAc (3×50 mL). The organics were combined, dried over $MgSO_4$, and concentrated in vacuo. Residual DMF was removed by co-stripping with Et_2O and mild heating in vacuo. Silica gel chromatography (hexane/CH_2Cl_2/MeOH 6:3:1), concentration in vacuo, and subsequent lyophilization from H_2O/CH_3CN afforded **59** (504 mg, 95 %) as a white solid. ^1H NMR ($CDCl_3$): $\delta = 9.68$ (br s, 1 H), 7.53–7.29 (m, 9 H), 4.98 (t, $J = 9.2$ Hz, 1 H), 4.91 (t, $J = 9.5$ Hz, 1 H), 4.84 (dd, $J = 9.9$, 9.1 Hz, 1 H), 4.32 (d, $J = 10.3$ Hz), 3.98 (**A**B, $J_{AB} = 16.4$ Hz, 1 H), 3.92 (**A**BX, $J_{AB} = 12.4$ Hz, $J_{AX} = 4.5$ Hz, 1 H), 3.82 (A**B**, $J_{AB} = 16.4$ Hz, 1 H), 3.61 (A**B**X, $J_{AB} = 12.4$ Hz, $J_{BX} = 1.5$ Hz, 1 H), 2.62 (AB**X**Y, $J_{XY} = 9.7$ Hz, $J_{XA} = 4.5$ Hz, $J_{XB} = 1.5$ Hz, 1 H), 2.02 (s, 3 H), 1.99 (s, 3 H), 1.96 (s, 3 H), 1.82 ppm (s, 3 H); ^{13}C NMR ($CDCl_3$): $\delta = 170.8$, 170.4, 169.4, 169.2, 152.6, 141.0, 140.8, 132.8, 130.8, 129.8, 129.1, 128.9, 128.1, 127.9, 127.7, 79.6, 75.2, 73.7, 69.8, 67.7, 61.5, 36.0, 20.9, 20.7, 20.6 ppm; HRMS (ESI): *m/z*: calcd for $[M+Na]^+$: 596.1566; found: 596.1573.

2,3,4,6-Tetra-*O*-acetyl-1-(biphenyl-2-yl)methyl glucosinolate (61): Anhydrous pyridine (8.25 mL, 8.07 g, 102.0 mmol) was dissolved in CH_2Cl_2 (8.9 mL), and the system was cooled to 0 °C. A solution of chlorosulfonic acid (740 μL, 1.29 g, 11.1 mmol) in CH_2Cl_2 (8.9 mL) was slowly added over 5 min. The resulting solution was warmed to ambient temperature, a solution of **59** (293 mg, 0.51 mmol) in CH_2Cl_2 (6.2 mL) was added, and the solution was stirred at ambient temperature for 18 h. A solution of $NaHCO_3$ (1.37 g, 16.31 mmol) in H_2O (15 mL) was slowly added, and solvents were concentrated in vacuo. The remaining solution was extracted with EtOAc (3×50 mL) and the combined organics were dried over Na_2SO_4 and concentrated in vacuo. Two-step silica gel chromatography (EtOAc/hexane/MeOH 6:3:1 to EtOAc/MeOH 4:1, $R_f = 0.00$ in EtOAc/hexane/MeOH 6:3:1), concentration in vacuo, and subsequent lyophilization from H_2O/CH_3CN afforded **61** (271 mg, 79 %) as a white solid. ^1H NMR (CD_3OD): $\delta = 7.60$–7.34 (m, 9 H), 5.00 (t, $J = 9.4$ Hz, 1 H), 4.82 (t, $J = 9.8$ Hz, 1 H), 4.73 (t, $J = 9.5$ Hz, 1 H), 4.35 (d, $J = 10.2$ Hz, 1 H), 4.13 (**A**B, $J_{AB} = 16.4$ Hz, 1 H), 3.95 (A**B**, $J_{AB} = 16.4$ Hz, 1 H), 3.89 (**A**BX, $J_{AB} = 12.5$ Hz, $J_{AX} = 4.9$ Hz, 1 H), 3.65 (A**B**X, $J_{AB} = 12.5$ Hz, $J_{BX} = 1.9$ Hz, 1 H), 2.47 (AB**X**, $J_{AX} = 4.9$ Hz, $J_{BX} = 1.9$ Hz, 1 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.93 (s, 3 H), 1.76 ppm (s, 3 H); ^{13}C NMR (CD_3OD): $\delta = 172.1$, 171.5, 171.1, 170.6, 159.3, 142.2, 141.9, 133.5, 131.8, 131.1, 130.2, 130.0, 129.2, 129.1, 128.8, 80.7, 76.3, 74.5, 70.7, 68.9, 62.6, 36.4, 20.8, 20.6, 20.6, 20.4 ppm; HRMS (ESI): *m/z*: calcd for $[M+Na]^+$: 698.0954; found: 698.0934.

1-(Biphenyl-2-yl)methyl glucosinolate (39): Compound **61** (106 mg, 0.16 mmol) was dissolved in anhydrous MeOH (2.05 mL), and NaOMe in MeOH (0.5 M, 155 μL, 0.08 mmol) was added. The solution was stirred at ambient temperature for 1 h, after which AcOH (500 μL) was added. Concentration in vacuo, followed by lyophilization from H_2O/CH_3CN (40:1), afforded **39** as a white solid in quantitative yield. ^1H NMR (CD_3OD): $\delta = 7.60$ (m, 9 H), 4.14 (m, 1 H), 4.08 (br s, 2 H), 3.39 (**A**BX, $J_{AB} = 12.3$ Hz, $J_{AX} = 3.6$ Hz, 1 H), 3.31–3.26 (m, 1 H), 3.17 (A**B**X, $J_{AB} = 12.3$ Hz, $J_{BX} = 2.1$ Hz, 1 H), 3.06 (m, 2 H), 2.24 ppm (AB**X**, $J_{AX} = 3.6$ Hz, $J_{BX} = 2.1$ Hz, 1 H); ^{13}C NMR (CD_3OD): $\delta = 161.5$, 142.5, 142.2, 134.2, 131.6, 130.8, 129.9, 129.7, 129.1, 128.8, 128.4, 83.2, 80.9, 79.1, 74.1, 70.1, 61.7, 37.2 ppm; HRMS (ESI): *m/z*: calcd for $[M+Na]^+$: 530.0531; found: 530.0521.

Calcein AM and Cell Titer-Glo cytotoxicity assays: All cell lines except NmuMG were maintained in RPMI medium 1640 supplemented with FBS (10 % *w/v*) and penicillin/streptomycin (PS, 100 units mL^{-1} and 100 $\mu g\,mL^{-1}$). NmuMG cells were maintained in DMEM supplemented with FBS (10 % *w/v*), insulin (10 $\mu g\,mL^{-1}$), and penicillin/streptomycin (PS, 100 units mL^{-1} and 100 $\mu g\,mL^{-1}$, respectively). Cells were harvested by trypsinization with trypsin (0.25 %) and EDTA (0.1 %) and were then counted in a hemocytometer in duplicate with better than 10 % agreement in field counts. Cells were plated at a density of 10 000–15 000 cells per well of each 96-well black tissue culture treated microtiter plate. Cells were grown for 1 h at 37 °C, with 5 % CO_2/95 % air in a humidified incubator to allow cell attachment to occur before compound addition. Library members were stored at −20 °C under desiccating conditions before the assay. Library member stocks (100×) were prepared in 96-well V-bottom polypropylene microtiter plates. Five serial 1:2 dilutions were made with anhydrous DMSO at 100× the final concentration used in the assay. The library-member-containing plates were diluted 1:10 with complete cell culture medium. The 10× stocks (10 μL) were added to the attached cells by Biomek FX liquid handler (Beckman Coulter). Library member stocks (10 μL) were added to cells (90 μL) in each plate to ensure full mixing of stocks with culture media by Biomek FK liquid handler with a 96-well head. Cells were incubated with the library members for 72 h before fluorescence reading. Test plates were removed from the incubator and washed once in sterile PBS to remove serum containing calcium esterases. Calcein AM (acetoxymethyl ester) reagent (30 μL, 1 M) was added, and the cells were incubated for 30 min at 37 °C. Plates were read for emission with a fluorescein filter (excitation 485 nm, emission 535 nm). An equal volume (30 μL) of cell titer-glo reagent (Promega Corporation, Inc.) was added, and the system was incubated for 10 min at room temperature with gentle agitation to lyse the cells. Each plate was reread for luminescence to confirm the inhibition observed in the fluorescent Calcein AM assay.

IC_{50} calculations: For each library member, at least three dose-response experiments were conducted on separate plates. For each experiment, percent inhibition values at each concentration were expressed as a percentage of the maximum emission signal observed for a 0 μM control. To calculate IC_{50} values, percent inhibitions were plotted as a function of log[concentration] and then fitted to a four-parameter logistic model that allowed for a variable Hill slope with use of XLFIT 4.1 (ID Business Solutions, Emeryville, CA). Range and mean IC_{50}s in human cancer cell lines and NmuMG cells are reported in Table 1. Average IC_{50}s in cancer cells were determined from the IC_{50} value for each cell line with the smallest standard error, excluding values reported as limits (see Supporting Information).

Preparation of isothiocyanate and glucosinolate stock standard solutions: Stock solutions of isothiocyanates **29** and **36** (50 mM) were prepared with HPLC-grade acetonitrile. Stock solutions of glucosinolates were prepared in distilled and deionized H_2O (ddH_2O) for **38** (46.3 mM), **39** (45.0 mM), and **62** (51.8 mM; see Figure 4 for structure); the stock solution of **62** was created from commercial sinigrin X-hydrate and standardized by UV/Vis spectroscopy with $\varepsilon_{227} = 6458\,M^{-1}\,cm^{-1}$.[25] For enzyme assays, glucosinolate stocks were each diluted to 10 mM in ddH_2O to retain consistency.

Determination of myrosinase specific activity: Myrosinase stocks were made from commercially available myrosinase isolated from *Sinapis alba* seeds (Sigma–Aldrich) in ddH_2O at a final concentration of 10 $mg\,mL^{-1}$. According to Sigma–Aldrich, enzyme specific activity was 0.361 $U\,\mu g^{-1}$, with one unit defined as the amount of enzyme able to hydrolyze 1 nmol **62** min^{-1} at 25 °C and pH 6.0. Stock solutions were calibrated for specific activity by measurement of the decrease in absorbance at 227 nm in 10 mm pathlength quartz cells on a Hitachi U-3000 recording spectrophotometer fitted with a PolyScience Model 9100 Refrigerated Constant Temperature Circulator. Each final reaction mixture contained sinigrin in ddH_2O (51.8 mM, 5 μL) and myrosinase stock (0.91 $U\,\mu L^{-1}$ in

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ddH$_2$O, 0–6 μL) in phosphate buffer (pH 7.4, 0.1 M) with a total volume of 1.000 mL. Solutions of sinigrin in phosphate buffer (0.1 M) were stabilized at 37 °C for 10 min prior to addition of enzyme and initiation of the reaction. Linear regression of absorbance values converted into the corresponding concentrations ($\varepsilon_{227} = 6458\,\text{M}^{-1}\,\text{cm}^{-1}$) over the first 1000 sec provided reaction rates as a function of enzyme concentration. A unit of myrosinase activity was defined as the amount of enzyme that catalyzed the hydrolysis of 1 nmol sinigrin min^{-1} at pH 7.4 and 37 °C. The specific activities of myrosinase stocks were calculated from the linear regression of reaction rates as a function of enzyme concentration.

Spectrophotometric assay for myrosinase activity: Myrosinase digestion of sinigrin was monitored by following the linear absorbance decrease at 227 nm and 37 °C in 10 mm pathlength quartz cells on a Hitachi U-3000 recording spectrophotometer fitted with a PolyScience Model 9100 Refrigerated Constant Temperature Circulator. Five serial 1:2 dilutions of sinigrin stock (51.8 mM in ddH$_2$O) were made. Each 1.000 mL assay reaction mixture contained a sinigrin solution (5 μL), myrosinase stock (2.74 U, 3 μL), and phosphate buffer (pH 7.4, 0.1 M, 992 μL). The buffered substrate was incubated at 37 °C for 10 min prior to enzyme addition. K_m and V_{max} values were determined by Lineweaver–Burk plotting of linear reaction rates over the initial 3 min with a suitable range of substrate concentrations (0.015–0.26 mM).

General RP-HPLC methodology: Analytical HPLC separation was performed on a Waters gradient-controlled HPLC system (Milford, MA) fitted with a Waters diode array detector (model 996). HPLC-grade acetonitrile and ddH$_2$O mobile phases contained TFA (0.1 %); methanol was HPLC grade and did not contain TFA. Analysis of samples was carried out on an Agilent Zorbax 300SB-C18 reversed-phase analytical column (3.5 μm, 50 mm × 4.6 mm) at 28 °C with a flow rate of 1 mL min^{-1} (ddH$_2$O w/0.1 % TFA at pump A, acetonitrile w/0.1 % TFA at pump B, and methanol at pump C). A linear gradient program was used, starting at 0.01 min from 0 % pump C to 90 % pump C over 60 min with a constant 5 % pump B. After each gradient run, the column was equilibrated at a flow rate of 1 mL min^{-1} 95 % pump A, 5 % pump B for 30 min.

Standardization of integrated areas in RP-HPLC injections: Stock solutions of compounds **29**, **36**, **38**, and **39** were diluted to 1 mM in phosphate buffer (PB, 0.1 M, pH 7.4). An equivalent volume of HPLC-grade acetonitrile was added to each of these solutions, to give a final concentration of 0.5 mM in CH$_3$CN/PB 1:1. The stock solution of **62** was diluted to 1 mM in PB without further addition of acetonitrile. For each of these standard solutions, a minimum of seven injections were performed, with sample injection volumes ranging between 10–100 μL.

Each resulting diode array spectra was corrected by subtraction from an appropriate solvent-only injection spectrum consisting either of PB (50 μL) or CH$_3$CN:PB 1:1 (50 μL). For each compound injection spectrum, the chromatogram at 227 nm was extracted and the area under the curve between standard retention times was calculated—**29**: 42.0 to 56.0 min; **36**: 40.0 to 54.0 min; **38**: 0.6 to 1.8 min and 8.0 to 28.0 min; **39**: 0.6 to 1.8 min and 8.0 to 28.0 min; **62**: 0.6 to 2.0 min. The average integration area over the same appropriate retention time resulting from the spectra of three baseline-corrected, solvent-only injections was subtracted from each of these areas. For each compound, the relationship between the amount of compound injected and the baseline-corrected peak area was linear between 5.0 and 50.0 nmol, with linear correlation coefficients (r^2) ranging from 0.9923 to 0.9993. The corresponding UV/Vis spectra for all compound injections can be found in the Supporting Information.

Determination of myrosinase-dependent hydrolysis rates by RP-HPLC: Hydrolysis reactions with **62** were performed in triplicate in Waters 1 mL clear glass vials with caps. PB (898 μL) and **62** in ddH$_2$O (10 mM, 100 μL) were added to each vial, to give an initial reaction concentration of 1 mM. Vials were equilibrated at 37 °C for 10 min prior to addition of myrosinase stock (2.27 U, 2 μL). Injections (50 μL) were performed by autosampler immediately after addition of enzyme and after every 1.5 h until 9.0 h.

Hydrolysis reactions with **38** and **39** were performed in triplicate in 1.5 mL flat-top microcentrifuge tubes (Fisher Scientific), with a final volume of 250 μL and an initial glucosinolate concentration of 1 mM. PB (2.200 mL) and **38** or **39** in ddH$_2$O (10 mM, 250 μL) were placed in a separate, larger vial; this solution (245 μL) was added to each of eight microcentrifuge tubes. An additional tube containing PB (245 μL) was included as a negative control. Microcentrifuge tubes were incubated at 37 °C with use of a VWR standard heatblock for 10 min prior to addition either of myrosinase stock (0.37 U μL^{-1} for reactions with **38**, 0.91 U μL^{-1} for reactions with **39**, 5 μL) or PB (24 h null myrosinase control, 5 μL). At each timepoint (0, 2, 4, 6, 8, 10, 24 h), HPLC-grade acetonitrile (250 μL) was added and the contents of the tube were vortexed for 10 s and transferred to a Waters 1 mL clear glass vial, and an injection (10 μL) was made by the autosampler.

Concentrations of **29**, **36**, **38**, **39**, and **62** in the samples were calculated from the generated standard curves. At each timepoint, the average concentration and standard deviation was calculated with use of a minimum of three replicate trials. Rates of hydrolysis for **38**, **39**, and **62** were determined from the decrease in average concentration over time, and were corrected for the amount of myrosinase used in the assay.

Determination of isothiocyanate decomposition rates by RP-HPLC: Experiments assessing the stabilities of isothiocyanates **29** and **36** to aqueous buffer were performed in 1.5 mL flat-top microcentrifuge tubes (Fisher Scientific) in triplicate, with a final volume of 250 μL and an initial isothiocyanate concentration of 1 mM. Each reaction mixture contained phosphate buffer (0.1 M, pH 7.4, 245 μL) and was incubated at 37 °C (VWR standard heatblock) for 10 min prior to addition either of isothiocyanate stock (50 mM in CH$_3$CN, 5 μL) or PB (22 h null-isothiocyanate control, 5 μL). At each timepoint (0, 2, 4, 6, 8, 22 h), HPLC-grade acetonitrile (250 μL) was added, and the contents of the tube were vortexed for 10 s and transferred into a Waters 1 mL clear glass vial, and an injection (50 μL) was made by the autosampler. Concentrations of **29** and **36** in the samples were calculated from the generated standard curves. At each timepoint, the average concentration and standard deviation was calculated from a minimum of three replicate trials.

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