JOC The Journal of Organic Chemistry

Article

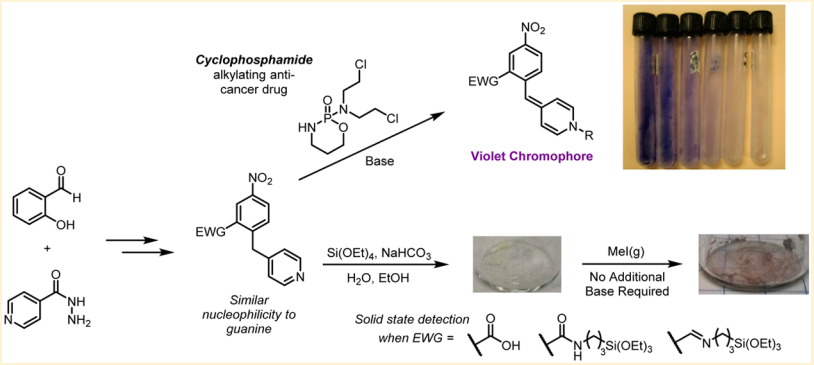
pubs.acs.org/joc

Synthesis and Performance of a Biomimetic Indicator for Alkylating Agents

Philip A. Provencher and Jennifer A. Love*

Department of Chemistry, The University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada

Supporting Information



ABSTRACT: 4-(4-Nitrobenzyl)pyridine (NBP) is a colorimetric indicator compound for many types of carcinogenic alkylating agents. Because of the similar reactivity of NBP and guanine in DNA, NBP serves as a DNA model. NBP assays are used in the toxicological screening of pharmaceutical compounds, detection of chemical warfare agents, environmental hygiene technology, preliminary toxicology tests, mutagenicity of medicinal compounds, and other chemical analyses. Nevertheless, the use of NBP as a DNA model suffers from the compound's low water solubility, its lack of reactive oxygen sites, and dissimilar steric encumbrance compared to DNA. We report herein the design and synthesis of NBP derivatives that address some of these issues. These derivatives have been tested in solution and found to be superior in the colorimetric assay of the alkylating anticancer drug cyclophosphamide. The derivatives have also been integrated into a polymeric silica material which changes color upon the exposure to dangerous alkylating agents, such as iodomethane vapor, without the need for an exogenous base. This material modernizes the NBP assay from a time-consuming laboratory analysis to a real-time solid state sensor, which requires neither solvent nor additional reagents and can detect both gas- and solution-phase alkylating agents.

INTRODUCTION

Alkylating agents are broadly used as active pharmaceutical ingredients (APIs), agrochemicals, industrial and laboratory reagents and solvents, and chemical warfare agents. Alkylating agents can be highly toxic, mutagenic, and/or carcinogenic because they form covalent bonds with endogenous nucleophiles, including DNA and proteins.[1] In fact, covalent interactions of drugs have been the oft-cited reason for idiosyncratic drug toxicity.[2]

Moreover, chronic exposure of hospital and pharmacy workers to alkylating agents has been discovered to pose a major problem for the healthcare industry. A recent study indicated that over 60% of surfaces sampled in hospital pharmacies had unsafe levels of cytotoxic drugs, including cyclophosphamide (CP), which is a commonly used alkylating anticancer drug.[3,4] A number of other studies indicated similar problems worldwide at facilities where cytotoxic drugs are handled.[5] Given that dermal penetration is one of the major mechanisms for exposure to cytotoxic drugs,[6] these data point to a widespread problem in hospitals and pharmacies. To date, there is no fast, effective method for detecting surface contamination with anticancer agents.

In the past ten years, there has been significant research in the synthesis of sensors that can detect trace levels of alkylating agents.[7] An ideal sensor would incorporate a number of beneficial properties, including (1) "turn-on" sensing,[7b] (2) biologically relevant limits of detection,[7b] (3) insensitivity to Brønsted acids and polar solvents,[7a,d] and (4) robustness toward air oxidation and other degradation pathways.[7e] Many of the recent published sensors do not achieve all of these properties.

We sought to develop a biomimetic sensor for the detection of alkylating agents that meets these specifications. Such a sensor has a number of potentially applications, including detection of contamination in hospitals and incorporation into a badge dosimeter to monitor long-term exposure of workers to alkylating agents. The design is based on the 4-(4-nitrobenzyl)-pyridine (NBP, **1**, Figure 1) scaffold. NBP is a colorimetric

Received: July 9, 2015
Published: September 22, 2015

DOI: 10.1021/acs.joc.5b01584
J. Org. Chem. 2015, 80, 9603−9609

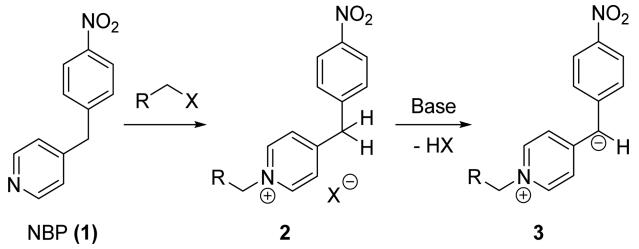


Figure 1. Mechanism of sensing by NBP.

indicator that detects alkylating agents at nanomolar levels. Guanine and NBP have been shown to have very close Swain–Scott nucleophilicity values,[10] and the pyridine functionality has similar nucleophilicity to the *N*7 position of guanine. Upon alkylation, NBP is susceptible to deprotonation.[8] The resulting delocalized carbanion **3** has an extremely dark violet coloration and can be measured by UV–vis spectroscopy at 575 nm absorbance.[9]

In addition, the reactivity of NBP in the ring opening of epoxides correlates to the mutagenic potency of the epoxide.[10,11] For these reasons, NBP has been proposed to be an effective model of DNA.[12] Accordingly, assays using NBP have been used to predict the *in vivo* reactivity of electrophilic drugs. NBP is not sensitive to the presence of Brønsted acids, polar solvents, or metal ions, which are common interferents in other alkylating agent sensors.[7a,d,f] The challenges associated with the use of NBP as a sensor include low solubility, the need for an exogenous base to generate the carbanion, and poor stability of the dye. We anticipated that the incorporation of an electron-withdrawing group onto the NBP scaffold would improve all of these characteristics and would also permit the incorporation of an NBP derivative into materials that could be used as a solid state sensor.

We elected to first explore installation of a carbonyl functionality on the nitrobenzene ring (**4**, Figure 2). This

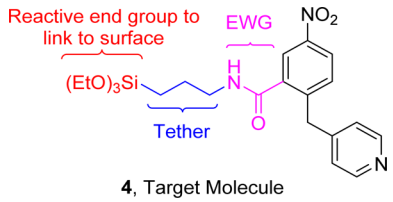


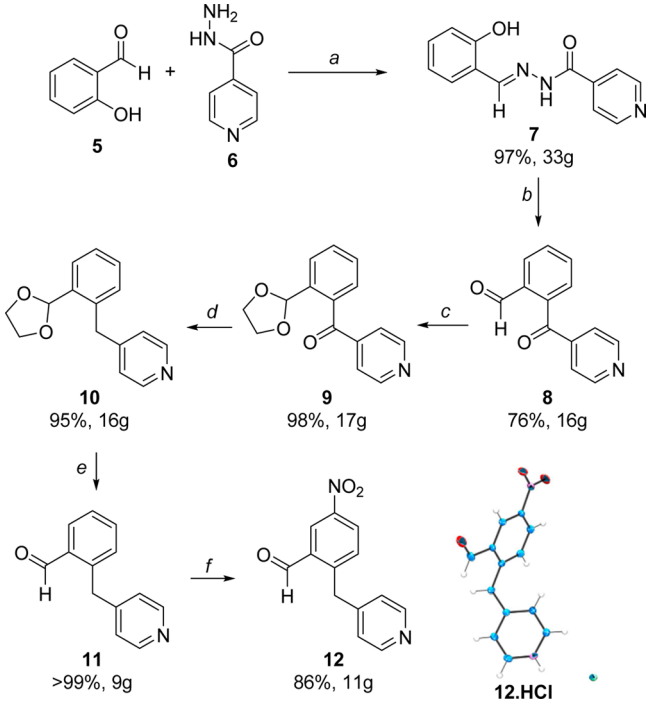
Figure 2. Target molecular sensor.

modification would increase the acidity of the sensor upon alkylation, increasing the rate of response to an alkylating agent and potentially obviating the need for an exogenous base. A carbonyl functionality could also potentially improve NBP's ability to mimic the nucleophilic reactions of guanine. While *N*7-alkylation of guanine in DNA is the most cited mode of cytotoxicity caused by alkylating agents,[13] other reports indicate that *O*6-alkylation of guanine and *O*4-alkylation of thymine in DNA are the major adducts that lead to cancer.[14] We anticipated that a sensor bearing a carbonyl functionality might also detect electrophiles capable of *O*-alkylation. We thus targeted a number of functional groups that could all be obtained from a common aldehyde intermediate. In addition, **4** contains a siloxane functionality that could permit impregnation into a sol–gel. We theorized that a silica material formed by base catalysis could facilitate the proton removal as necessary for the dye formation.

RESULTS AND DISCUSSION

Synthesis of Proposed Molecular Sensor. A target molecule of type **4** could, in principle, be prepared by a number of methods. We elected to pursue the strategy outlined in Scheme 1, which allowed the synthesis of aldehyde **12** on a

Scheme 1. Attaining Key NBP Intermediate by LTA Induced Oxidative Rearrangement[a,b]



[a]Reagents: (a) EtOH; (b) $Pb(OAc)_4$, THF; (c) ethylene glycol, toluene, *p*-TsOH; (d) N_2H_4, KOH; (e) 3 M HCl; (f) HNO_3, H_2SO_4. [b]Masses next to yields indicate largest scale performed of each reaction, in grams of isolated product.

multigram scale in six steps with an overall yield of 59%. Inexpensive starting materials **5** and **6** were condensed to provide acylhydrazone **7** in near-quantitative yield. Using a known oxidative rearrangement, **7** was converted to dione **8**.[15] This was the only step in the synthesis that required column chromatography. Acetal protection of the aldehyde moiety allowed for the subsequent deoxygenation of the ketone in **9** by Wolff–Kishner reduction, as has been described previously for the deoxygenation of diarylketones.[16] After removal of the acetal protecting group, subsequent nitration of **11** led to **12**. It is notable that **12** proved to be quite sensitive to both bases and electrophilic solvents, in support of our hypothesis about the effect of incorporating the carbonyl functionality. The use of ethyl acetate as the solvent and the slow addition of sodium bicarbonate during workup phase allowed for **12** to be obtained in high yields.

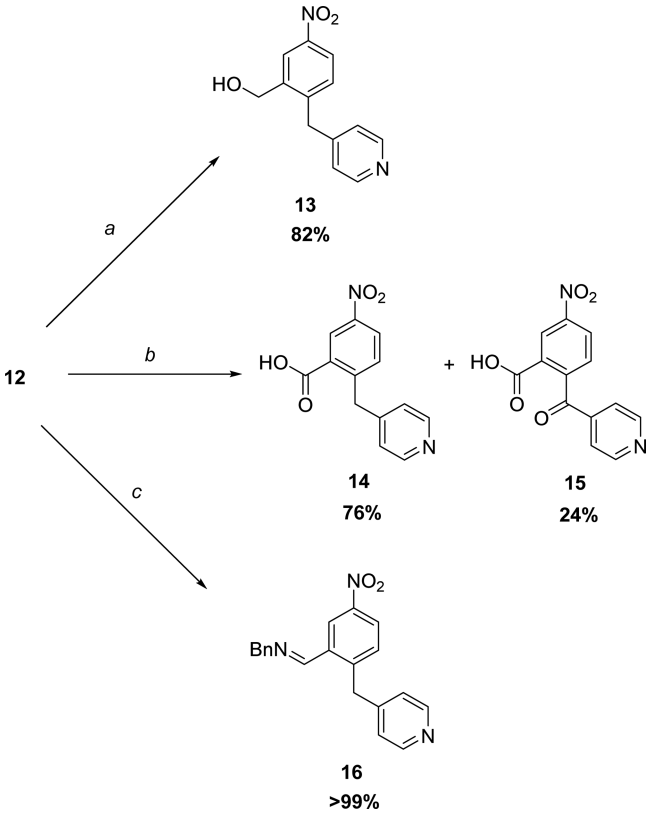
A single crystal of **12·HCl** was grown from the slow generation of HCl in chloroform, and its ORTEP is shown in Scheme 1. The use of trimethylsilyl chloride and imidazole resulted in immediate dye formation and rapid decomposition and precipitation. In contrast, the use of *tert*-butyldiphenylsilyl chloride and imidazole gave the favorable slow formation of

DOI: 10.1021/acs.joc.5b01584
J. Org. Chem. 2015, 80, 9603–9609

crystalline material, presumably because the greater steric hindrance of TBPSCl prevented silylation at the pyridine nitrogen. The HCl-adduct of NBP was also crystallized by this method. **12·HCl** has similar bond lengths and angles as reported in related structures.[17]

Effect of Functional Group Modification on Sensing Capability. Aldehyde **12** was converted into a number of other NBP derivatives (Scheme 2). Reduction to alcohol **13** proceeded smoothly by treatment with sodium borohydride in ethanol and precipitation of the product. Oxidation to carboxylic acid **14** was not as straightforward. Initial tests of Pinnick oxidation conditions yielded a 1:1 mixture of desired acid **14** and overoxidized product **15**. Though benzylic oxidations are known to occur with sodium chlorite, they generally require an additional oxidant such as *tert*-butylhydroperoxide or a transition metal catalyst.[18] After optimization, the desired product was obtained in 76% yield. Imine **16** was obtained in quantitative yield by treatment of **12** and benzylamine with magnesium sulfate in ethyl acetate at ambient temperature.

Scheme 2. Derivatization of NBP Moiety 12[a]



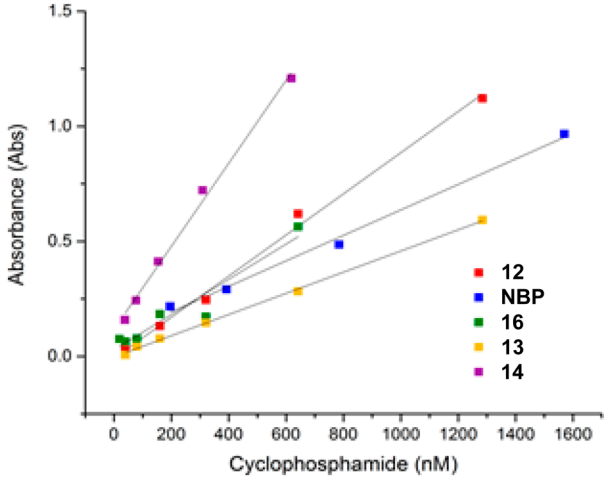
[a]Reagents: (a) $NaBH_4$, EtOH; (b) $NaClO_2$, NaH_2PO_4, H_2O, acetone, 2-methyl-2-butene; (c) $BnNH_2$, $MgSO_4$, EtOAc.

With the series of NBP derivatives in hand, we next sought to compare their activity under standard assay conditions for the detection of commonly used chemotherapeutic cyclophosphamide. Our focus on cyclophosphamide stems from recent studies showing that hospital environments are contaminated with this potent carcinogen, and so the real-time detection of cyclophosphamide would be advantageous.[19] The specific assay method from literature was chosen because it did not require liquid/liquid extraction of the chromophore or the avoidance of light.[20]

Assay Development. A 105−6740 nM calibration curve of cyclophosphamide in water was generated by serial dilutions, with all samples being 1 mL. Three control samples without cyclophosphamide were also used. The samples were then cooled to 0 °C. A 1 mL aliquot of 0.2 M NaOAc buffer pH 4.5 was then added. We then added 0.75 mL of 3.3% (w/v) **NBP** or derivative **12**, **13**, **14**, or **16**. While the method described by Christian et al. utilized acetone as the solvent for the 3.3% stock solution of NBP, in this work the solvent was altered due to the differing solubilities of the NBP derivatives. For NBP, aldehyde **12**, and benzyl imine derivative **16**, acetone was used as the solvent. For the carboxylic acid derivative **14**, aqueous KOH solution was used. Using water as the sole solvent during the course of the reaction potentially increases the similarity of the assay conditions to the biological system; a previous report indicates that the commonly used aqueous−organic mixtures furnish a significantly different chemical environment compared to *in vivo* conditions.[12] For alcohol **13**, *N*,*N*-dimethylformamide was used as the solvent. The samples were heated to 100 °C for 20 min in closed vials. After cooling samples to room temperature, 2.5 mL of 1:1 triethylamine/acetone were added. Samples were shaken by a hand mixer for 30 seconds, and scan mode UV/vis spectra were taken within an hour. The average of the three blanks were calculated and subtracted from all of the samples. The sample coloration was stable for months at −20 °C, but degraded within a week at room temperature.

The results of the assays with NBP, **12**, **13**, **14**, and **16** for the trace detection of cyclophosphamide are shown in Chart 1 and Table 1. The molar absorptivities of the dyes resulting from reaction of the NBP series with CP followed the trend **14** > **12** ≈ **16** > **NBP** > **13**. As the dye became more intense, the absorption wavelength also lengthened. Unsurprisingly, the more electron-rich alcohol derivative **14** has the least intense dye, consistent with an expected decrease in pK_a relative to NBP. The carboxylate derivative **14** yielded the most intense dye, with the longest absorption wavelength. The carboxylate

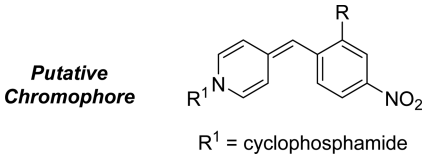
Chart 1. Trace Cyclophosphamide Determination by NBP and Its Derivatives[a]



[a]The concentration of cyclophosphamide on the abscissa axis is recorded as the actual dilution of the compound after all of the assay reagents were added.

DOI: 10.1021/acs.joc.5b01584
J. Org. Chem. 2015, 80, 9603−9609

Table 1. Absorption Values of NBP Derivatives in Their Dye Form



R^1 = cyclophosphamide

Entry	R	ε (L mol^{-1} cm^{-1})	λ_{anal} (nm)	Linear Fit (R^2)	Relative Dye Intensity
1	CH_2OH	0.46×10^6	570	0.998	0.84
2	H	0.55×10^6	575	0.991	1.0
3	CHNBn	0.77×10^6	581	0.899	1.4
4	CHO	0.89×10^6	587	0.994	1.6
5	COOK	1.8×10^6	603	0.992	3.3

derivative **14** yields a dye over three times more intensely colored than the dye produced from NBP. The stability of the dyes over time followed the same trend as the color intensity, with the dye forms of carboxylic acid **14** being the most stable (over six months in DMSO when treated with iodomethane) and the alcohol **13** being the least stable. None of the tested dyes showed any sensitivity to Lewis acidic metal ions such as Sc(III) triflate or zinc acetate, and strong acids did not yield any response. As mentioned previously, these are common interferents in other sensors of alkylating agents.[7a,d,f] Buffers were not required for the color reaction to take place, though reactions gave the cleanest UV−vis spectra when buffers were utilized.

We then sought to prepare a siloxane-containing derivative of type **4**. We anticipated that carboxylic acid **14** could be coupled with an amine to yield amide **4**. The use of common amide bond forming reagents (thionyl chloride, carbonyldiimazole (CDI), and PyBOP) proved challenging because of the reactivity of **14**. Nevertheless, CDI was found to give the cleanest amide coupling reaction. Although amide **4** proved to be too unstable to isolate, a one-pot procedure involving sequential amide bond formation and incorporation into silica (tetraethoxysilane, ethanol/water, sodium carbonate catalyst) was successful. Presumably the trisethoxysilane moiety is covalently bound in the silica matrix in this way. Imine **17** and the potassium salt of **14** were also immobilized in silica matrices under the same base catalyzed silica forming conditions. The silica-based materials were isolated as opaque powdery solids.

We devised some simple experiments to demonstrate a proof-of-concept that these materials could detect alkylating agents. The procedure to test the materials was adapted from methods outlined by the Eichen and Rebek groups, wherein they simply allowed a volatile alkylating agent to diffuse through a porous material.[7a,b] In this way, the silica materials were exposed to iodomethane at an approximate concentration of 3 mg/m^3 (based on the iodomethane vapor pressure of 54.4 kPa). While this concentration of iodomethane is higher than the OSHA standard permissible exposure limit of 0.028 mg/L, it was the lowest concentration we were able to achieve.[21] As described in the Experimental Section, when the solids derived from the NBP analogs were exposed to iodomethane vapor, the materials underwent a slow color formation, with the silica material based on carboxylic acid **14** giving the deepest violet coloration without any base additive or heating. Surprisingly, the silica impregnated with imine **17** turned from an orange/

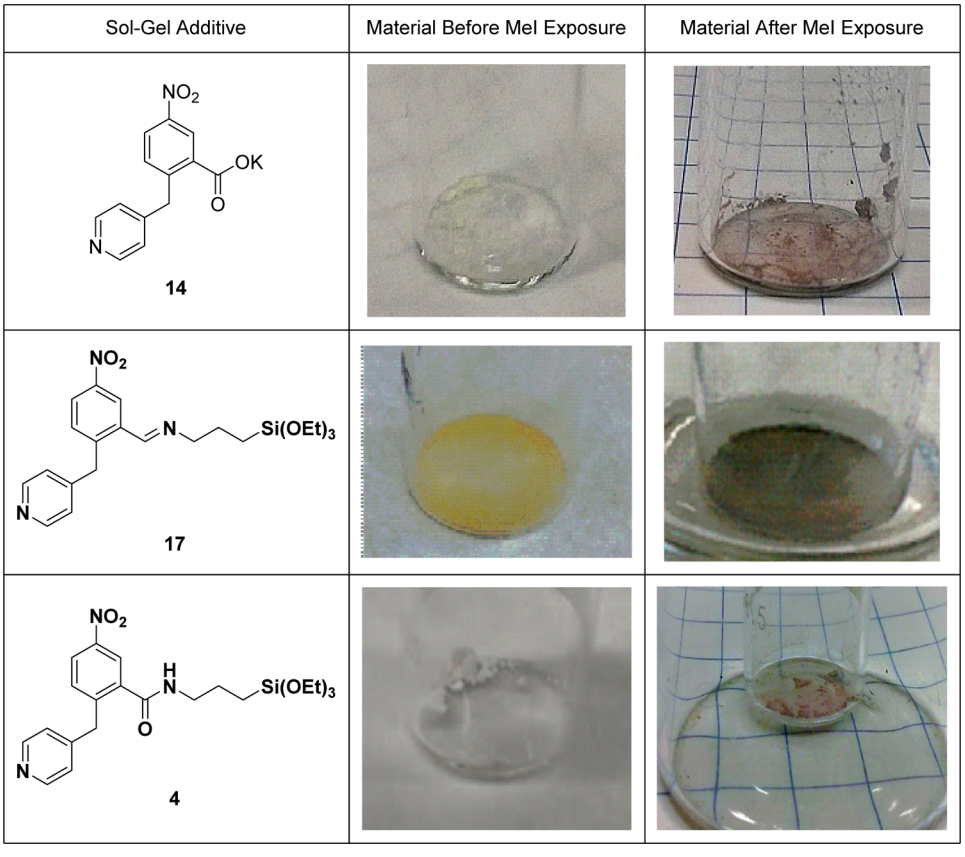


Figure 3. Responses of silica materials to iodomethane vapor after **14** hours exposure.

DOI: 10.1021/acs.joc.5b01584
J. Org. Chem. 2015, 80, 9603−9609

yellow material to a green material. Amide **4** yielded the poorest violet coloration, turning from a white powder into a light purple (Figure 3). Silica materials synthesized with base catalysis were able to sense iodomethane vapor without added base, whereas no sensing occurred if the impregnated silica material was prepared by acid catalysis. Thus, it appeared that the silica was able to facilitate proton transfer from the NBP derivatives once they were alkylated. The materials gave slow responses to the iodomethane vapor, with coloration appearing after an hour of exposure. Nevertheless, these results demonstrate that functionalization of NBP leads to viable sensors for alkylating agents.

CONCLUSION

In this work we have reported a robust, high yielding synthesis of novel NBP derivatives that serve as indicators for alkylating agents. The sensing capabilities of these compounds were tested in both solution assay and in the solid state. Of these synthesized NBP derivatives, the potassium salt of carboxylic acid derivative **14** was found to be the most efficacious in sensing alkylating agents both in solution and in the solid state. The potassium salt of **14** has significant advantage over state-of-the-art compound NBP. This compound is soluble in water, which is a greater mimic of *in vivo* conditions, while also producing a 3-fold stronger color when in the dye state. Containing a carbonyl, carboxylic acid **14** and amide **4** have the potential to sense *O*-alkylating agents in addition to *N*-alkylating agents. Finally, the potassium salt of **14** may be impregnated directly into a sol−gel as a solid state sensor. These indicators have potential use in assay and sensor applications for detection of alkylating agents in hospitals and pharmacies. Efforts to improve the response time are underway.

EXPERIMENTAL SECTION

***N′*-(2-Hydroxybenzylidene)isonicotinohydrazide (7).** Isoniazid (20 g, 146 mmol), salicylaldehyde (15.5 mL, 146 mmol), and ethanol (700 mL) were combined in a round-bottom flask and heated to reflux overnight while stirring. The title compound was formed as a white precipitate, which was filtered and washed with chilled ethanol (33.14 g, 97%): Mp 240−242 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.29 (s, 1 H), 11.08 (s, 1 H), 8.80 (d, J = 5.8 Hz, 2 H), 8.68 (s, 1 H), 7.92−7.75 (m, 2 H), 7.62−7.57 (m, 1 H), 7.32 (s, 1 H), 6.99−6.87 (m, 2 H) ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6) δ = 161.3, 157.4, 150.4, 148.9, 140.0, 131.7, 129.2, 121.5, 119.4, 118.7, 116.4; HRMS (ESI+ TOF MS) m/z 242.0930 (calcd for $C_{13}H_{11}N_3O_2$ + H^+ 242.0930).

2-Isonicotinoylbenzaldehyde (8). Compound **7** (25 g, 103.7 mmol) was dissolved in dry THF (625 mL) in a 2 L flame-dried two-necked flask. Lead tetraacetate (50.5 g, 113.9 mmol) was added slowly under nitrogen while vigorously stirring the mixture, resulting in a strong effervescence. The vessel was then closed with a ground glass stopper, though kept open to the Schlenk line N_2 and bubbler in order to allow for escaping gas. The mixture was stirred for 3 h at room temperature. The THF was then removed under reduced pressure, and the mixture was dissolved in ethyl acetate. The resulting slurry was then filtered through a silica plug. The title compound was purified by gradient column chromatography (loading with 1:1 ethyl acetate/light petroleum ether and gradient to neat ethyl acetate) yielding a light yellow oil (16.6 g, 76%): R_F 0.21 (in 1:1 pet. ether/ethyl acetate). IR (ATR) 1678.81 cm^{-1} (overlapping C═O); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.02 (s, 1 H), 8.80 (d, 2 H, J = 5.8 Hz), 8.05−8.01 (m, 1 H), 7.79−7.74 (m, 2 H), 7.57−7.56 (m, 2 H, J = 4.4, 1.7 Hz), 7.53−7.48 (m, 1 H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ = 195.7, 190.5, 150.4, 142.7, 138.9, 135.2, 133.7, 131.6, 131.1, 128.6, 122.0; HRMS (ESI+ TOF MS) m/z 212.0711 (calcd for $C_{13}H_9NO_2$ + H^+ 212.0712).

(2-(1,3-Dioxolan-2-yl)phenyl)(pyridin-4-yl)methanone (9). Compound **8** (14.6 g, 69.2 mmol) was dissolved in toluene (511 mL). *p*-Toluenesulfonic acid (0.4 g, 2.1 mmol) and distilled ethylene glycol (8.76 mL, 157 mmol) were then added to the solution. The solution was heated to reflux overnight with a Dean−Stark apparatus installed. After the reaction cooled to room temperature, saturated aqueous sodium bicarbonate (50 mL) was added. The toluene layer was separated, washed with saturated aqueous sodium bicarbonate (50 mL), and washed with brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and subsequently filtered. Removal of toluene under reduced pressure afforded the title compound as a yellow oil (17.4 g, 98%): IR (ATR) 1675.73 cm^{-1} (C═O); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.84−8.72 (m, 2 H), 7.70 (d, J = 7.5 Hz, 1 H), 7.60−7.53 (m, 3 H), 7.45 (t, J = 7.5 Hz, 1 H), 7.30 (d, J = 7.5 Hz, 1 H), 6.02 (s, 1 H), 3.91−3.80 (m, 4 H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ = 196.1, 166.0, 150.7, 143.3, 140.4, 132.8, 130.3, 130.2, 129.1, 127.7, 52.4, 1.0; HRMS (ESI+ TOF MS) m/z 256.0978 (calcd for $C_{15}H_{13}NO_3$ + H^+ 256.0974).

4-(2-(1,3-Dioxolan-2-yl)benzyl)pyridine (10). Compound **9** (17.7 g, 69.4 mmol) was dissolved in a minimum amount of methylene chloride (DCM) and transferred into a flame-dried double-walled Schlenk bomb under nitrogen gas. It is important that the flask be inspected carefully for cracks and in general must be in very good condition, as the reaction is performed under high pressure; additionally, the reaction must be performed behind a blast shield. The DCM was removed *in vacuo*, and distilled hydrazine (140 mL, 50−60% in H_2O) was added via syringe with a septum in place. The flask was sealed by a Teflon stopper, heated to 135 °C, and stirred vigorously overnight. Upon heating the starting material became soluble in the aqueous hydrazine mixture. The flask was then allowed to cool to room temperature while stirring under ambient conditions and opened under nitrogen gas. Powdered KOH (19.14 g, 341 mmol) was added; the tube was resealed and heated to 135 °C overnight with vigorous stirring. After the tube cooled, glass precipitate was observed, and to the reaction mixture was added 80:20 DCM/isopropanol (10 mL). Deionized water (10 mL) was added, and the mixture was extracted with DCM (3 × 100 mL). The organic layer was washed with brine and dried over anhydrous sodium sulfate, and the DCM was removed under reduced pressure to yield the title compound as a yellow oil, which was sufficiently pure to move on to the next step (15.9 g, 95%): IR (ATR) 1597.81 cm^{-1} (arom.); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.55−8.41 (m, 2 H), 7.65−7.59 (m, 1 H), 7.34−7.30 (m, 2 H), 7.14−7.09 (m, 1 H), 7.08 (d, J = 5.8 Hz, 2 H), 5.87 (s, 1 H), 4.17 (s, 2 H), 4.15−3.97 (m, 4 H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$) δ = 149.8, 139.8, 137.0, 135.6, 130.8, 129.4, 127.0, 126.7, 124.2, 101.9, 65.2, 37.3; HRMS (ESI+ TOF MS) m/z 242.1179 (calcd for $C_{15}H_{15}NO_2$ + H^+ 242.1181).

2-(Pyridin-4-ylmethyl)benzaldehyde (11). Aqueous 3 M HCl (561 mL) was added to compound **10** (11.5 g, 48 mmol) in a 1 L round-bottom flask and stirred at 50 °C for 3 h. After the reaction mixture was heated for 3 h, the heating bath was removed and the flask was allowed to cool to room temperature, at which temperature the reaction was stirred overnight. The reaction mixture was made basic by addition of KOH pellets while stirring. The mixture was extracted with DCM (4 × 60 mL), the organic layer was washed with brine and dried with anhydrous sodium sulfate, and the solvent was removed under reduced pressure to yield the title compound quantitatively as a bright green viscous oil, which was sufficiently pure to move on to the next step (9.4 g): IR (ATR) 1697.22 (C═O), 1597.49 (arom.) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.14 (s, 1 H), 8.49 (d, 2 H, J = 4.3 Hz), 7.87 (dd, 1 H, J = 7.5, 1.4 Hz), 7.61−7.48 ppm (m, 2 H), 7.29 (d, 1 H, 8.7 Hz), 7.07 (d, 2 H, 5.3 Hz), 4.46 (s, 2 H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ = 192.5, 149.7, 149.3, 140.3, 134.1, 133.95, 133.93, 131.9, 127.6, 124.0, 37.7; HRMS (ESI+ TOF MS) m/z 198.0916 (calcd for $C_{13}H_{11}NO$ + H^+ 198.0919).

5-Nitro-2-(pyridin-4-ylmethyl)benzaldehyde (12). Compound **11** (10.5 g, 53.3 mmol) was charged into a 350 mL round-bottom flask, and the flask was cooled to −10 °C in a dry ice/ethylene glycol bath. Concentrated H_2SO_4 (52.6 mL) was added to form a thick red tar. A 1:2 HNO_3/H_2SO_4 mixture (52.61 mL) was then slowly added,

DOI: 10.1021/acs.joc.5b01584
J. Org. Chem. 2015, 80, 9603−9609

and the reaction mixture was stirred overnight by spinning the flask mechanically in the cooling bath. The resulting orange solution was neutralized by dropwise addition of saturated aqueous sodium bicarbonate solution and periodically cooling the flask with cold water. Excessive foam from carbonate neutralization at this time required that the reaction be split into four 2 L round-bottom flasks. The resulting aqueous solutions were extracted individually with ethyl acetate (5 × 100 mL, each), the extracts were combined, and then the organic layers were washed with brine and dried over anhydrous sodium sulfate, followed by solvent removal under reduced pressure. The crude residue was taken up in chloroform and precipitated out by the addition of light petroleum ether to yield the product as an orange crystalline material. The solution (mother liquor) over the material was first removed by Pasteur pipet, and the residual solvent was removed from the solid product *in vacuo* (11.1 g, 86%): Mp 95−100 °C. IR (ATR) 1702.29 (C═O), 1599.72 (arom.), 1522.17 (NO_2, as) cm^{-1}; 1348.32 cm^{-1} (NO_2, s); ^1H NMR (300 MHz, $CDCl_3$) δ = 10.16 (s, 1 H), 8.63 (d, J = 2.3 Hz, 1 H), 8.44 (br., 2 H), 8.32 (dd, J = 2.5, 8.5 Hz, 1 H), 7.46 (d, J = 8.5 Hz, 1 H), 7.01 (d, J = 3.88 Hz, 2 H), 4.50 (s, 2 H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ = 190.1, 150.1, 147.4, 147.3 (overlapping), 147.2, 134.6, 133.1, 128.3, 127.9, 124.0, 37.6; HRMS (ESI+ TOF MS) m/z 243.0773 (calcd for $C_{13}H_{10}N_2O_3$ + H^+ 243.0770).

(5-Nitro-2-(pyridin-4-ylmethyl)phenyl)methanol (13). **12** (0.509 g, 2.1 mmol) was dissolved in ethanol (3 mL) in a 20 mL scintillation vial, and the solution was warmed to 40 °C while stirring. Solid $NaBH_4$ (0.154 g, 4.05 mmol) was added by spatula over 5 min, resulting in a black solution with some effervescence. The vial was capped and allowed to stir at 40 °C for 30 min, after which water (6 mL) was added to the reaction, which induced the precipitation of a yellow-orange solid. The solution was filtered off, and the solid was washed with cold water. Residual water was removed from the yellow material under vacuum in a desiccator to yield the title compound (0.42 g, 82%): Mp 134−140 °C IR (ATR) 3162 (OH), 1602 (arom.), 1510 (NO_2, as) cm^{-1}; 1342 cm^{-1} (NO_2, s); ^1H NMR (400 MHz, $CDCl_3$) δ = 8.50−8.46 (m, 2 H), 8.40 (d, J = 2.4 Hz, 1 H), 8.14 (dd, J = 2.4, 7.2 Hz, 1 H), 7.31 (d, J = 8.2 Hz, 1 H), 7.05 (d, J = 5.8 Hz, 2 H), 4.72 (s, 2 H), 4.14 (s, 2 H); $^{13}C\{^1H\}$ NMR (101 MHz, $(CD_3)_2CO$) δ = 151.2, 149.6, 148.6, 145.3, 144.2, 132.5, 125.5, 123.2, 122.9, 62.0, 38.2; HRMS (ESI TOF MS) m/z 245.0932 (calcd for $C_{13}H_{12}N_2O_3$ + H^+ 245.0926).

5-Nitro-2-(pyridin-4-ylmethyl)benzoic Acid (14). In a 50 mL round-bottom flask, 1.033 g of **12** (4.3 mmol) was combined with 2-methyl-2-butene (1.3 mL, 12.3 mmol) in 18 mL of acetone. $NaClO_2$ (0.7263 g, 8 mmol) and $NaH_2PO_4{\cdot}H_2O$ (4.6 g, 33.3 mmol) were dissolved in H_2O (8 mL) by sonication. The solution of $NaClO_2$ and $NaH_2PO_4{\cdot}H_2O$ was added to the acetone solution of **12** by Pasteur pipet, and this mixture was stirred at room temperature for 45 min. A white precipitate formed and was filtered and washed with acetone and water (0.843 g, 76%): Mp 223−226 °C. IR (ATR) 3084 (OH), 1708 (C═O), 1612 (arom.), 1520 (NO_2, as) cm^{-1}; 1346 cm^{-1} (NO_2, s); ^1H NMR (300 MHz, DMSO-d_6) δ = 8.56 (d, 1 H, J = 2.6 Hz), 8.43 (d, 2 H, J = 5.5 Hz), 8.27 (dd, 1 H, J = 8.5, 2.4 Hz), 7.17 (d, 2 H, J = 5.9 Hz), 4.52 (s, 2 H). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ = 167.5, 149.5, 149.1, 146.8, 146.0, 134.3, 133.0, 125.4, 124.9, 124.1, 37.7; HRMS (ESI+ TOF MS) m/z 259.0719 (calcd for $C_{13}H_{10}N_2O_4$ + H^+ 259.0719); Anal. Calcd for $C_{13}H_{10}N_2O_4$: C, 60.47; H, 3.90; N, 10.85. Found: C, 60.12; H, 3.93; N, 10.46.

***N*-Benzyl-1-(5-nitro-2-(pyridin-4-ylmethyl)phenyl)-methanimine (16).** Prepared in the same manner as **17** from **12** and benzylamine. The isolated material was a dark brown oil, and its purity as observed by ^1H NMR spectroscopy was deemed sufficient to use for assay (2.05g, >99%): IR (ATR) 1640 (C═N), 1599 (arom.), 1521 (NO_2, as), 1345 cm^{-1} (NO_2, s); ^1H NMR (300 MHz, $CDCl_3$) δ = 8.76 (d, J = 2.1 Hz, 1 H), 8.53 (s, 1 H), 8.48 (d, J = 5.0 Hz, 2 H), 8.23 (dd, J = 2.1, 8.2 Hz, 1 H), 7.42−7.28 (m, 4 H), 7.20 (d, J = 7.1 Hz, 2 H), 6.96 (d, J = 5.3 Hz, 2 H), 4.80 (s, 2 H), 4.40 (s, 2 H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ = 158.0, 150.1, 148.3, 147.4, 144.9, 138.4, 135.9, 132.5, 128.8, 128.2, 127.4, 124.8, 124.4, 124.0, 65.5, 38.1; HRMS (ESI+ TOF MS) m/z 332.1395 (calcd for $C_{20}H_{17}N_3O_2$ + H^+ 332.1399).

1-(5-Nitro-2-(pyridin-4-ylmethyl)phenyl)-*N*-(3-(triethoxysilyl)propyl)methanimine (17). A flame-dried flask was charged with **12** (105 mg, 0.43 mmol), ethyl acetate (1.68 mL), and anhydrous $MgSO_4$ (105 mg, 0.88 mmol). 3-Aminopropyltriethoxysilane (100.8 μL, 0.43 mmol) was added via micropipette, the vial was closed, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was then filtered through a plug of anhydrous sodium sulfate, and the solvent was removed under reduced pressure to yield the title compound quantitatively as a dark brown oil (191 mg, >99%): ^1H NMR (300 MHz, $CDCl_3$) δ = 8.71 (d, J = 2.3 Hz, 1 H), 8.52 (d, J = 5.5 Hz, 2 H), 8.47 (s, 1 H), 8.20 (dd, J = 2.4, 8.3 Hz, 1 H), 7.35 (d, J = 8.5 Hz, 1 H), 7.02 (d, J = 5.7 Hz, 2 H), 4.40 (s, 2 H), 3.83 (q, J = 7.1 Hz, 6 H), 3.60 (t, J = 6.76 Hz, 2 H), 1.87−1.73 (m, 2 H), 1.23 (t, J = 7.0 Hz, 9 H), 0.60−0.66 (m, 2 H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ = 157.0, 150.1, 148.0, 147.3, 144.5, 135.9, 132.1, 124.4, 124.2, 123.8, 64.7, 58.4, 37.8, 24.2, 18.3, 8.1; HRMS (ESI+ TOF MS) m/z 446.2115 (calcd for $C_{22}H_{31}N_3O_5Si$ + H^+ 446.2111)

*General Method for the Synthesis of Silica Sensing Materials Based on **14** and **17**.* TEOS (666 μL, 2.85 mmol) was combined with 2 mL of 0.15 M sodium carbonate solution in water. This mixture was stirred at room temperature until the two phases formed a suspension. **14** (74 mg, 0.285 mmol) was dissolved in 0.6 mL of saturated sodium bicarbonate solution by the addition of 200 μL of saturated sodium hydroxide solution with stirring. **17** (127, 0.285 mmol) was dissolved in 0.5 mL of ethanol. The solution of either **14** or **17** was then transferred into a mixture of TEOS and sodium carbonate. In the formation of silica material based on **14**, 0.5 mL of ethanol was also added. The reaction mixture was then stirred overnight, and the resulting white or lightly colored precipitate was collected by filtration.

*Method for the Preparation of Silica Sensing Material Based on **4**.* A flame-dried round-bottom flask fitted with a stir bar and cooled under $N_2(g)$ was charged with 50 mg of compound **14** (0.194 mmol) and 2 mL of dry DMF. The flask was cooled to −15 °C in a benzyl alcohol/dry ice bath, and carbonyldiimidazole was added (31.4 mg, 0.194 mmol) while stirring, immediately forming a dark orange color. The reaction mixture was stirred for 1 h at this temperature. 3-Aminopropyltriethoxysilane (45.5 μL, 0.194 mmol) was added via micropipette, and the reaction mixture was allowed to stir at room temperature for 1 h. The silica sensing material was formed directly by transferring this reaction mixture via cannula into a suspension of TEOS and sodium carbonate solution prepared as above. The resulting solution was stirred overnight, and the resulting white precipitate was collected by filtration.

The silica materials were assessed for their sensing ability by charging a 4 mL vial with 50 mg of the silica material and placing the vial into a 20 mL vial containing 1 mL of iodomethane. The larger vial was sealed with a cap and left to sit at room temperature.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01584.

Materials and methods, spectra (PDF)
X-ray crystallography (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: lovej@mail.ubc.ca.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the University of British Columbia, NSERC (Discovery grant, Research Tools and Instrumentation grants) and the Canada Foundation for Innovation for supporting this

DOI: 10.1021/acs.joc.5b01584
J. Org. Chem. 2015, 80, 9603−9609

research. Thanks go to Dr. Brian Patrick for X-ray crystallographic analysis, Dr. Maria Ezhova for assistance and training in NMR, and Dr. Yun Ling for mass spectrometry assistance.

REFERENCES

(1) (a) Reddy, L. R.; Corey, E. J. *Tetrahedron Lett.* **2005**, *46*, 927−929. (b) For a review of toxicities induced by off-target covalent interactions, see: Liebler, D. C.; Guengerich, F. P. *Nat. Rev. Drug Discovery* **2005**, *4*, 410−420.

(2) Notwithstanding the potential for toxicity, the covalent modification of biomolecules can be a powerful approach to the treatment of a wide variety of diseases, and indeed there has been a significant push to develop new "covalent drugs" in recent years. See: (a) Singh, J.; Petter, R. C.; Baillie, T. A.; Whitty, A. *Nat. Rev. Drug Discovery* **2011**, *10*, 307−317. (b) Zhou, S.; Chan, E.; Duan, W.; Huang, M.; Chen, Y.-Z. *Drug Metab. Rev.* **2005**, *37*, 41−213. (c) Kalgutkar, A. S.; Dalvie, D. K. *Expert Opin. Drug Discovery* **2012**, *7*, 561−581. (d) Kathman, S. G.; Xu, Z.; Statsyuk, A. V. *J. Med. Chem.* **2014**, *57*, 4969−4974. (e) London, N.; Miller, R. M.; Krishnan, S.; Uchida, K.; Irwin, J. J.; Eidam, O.; Gibold, L.; Cimermančič, P.; Bonnet, R.; Shoichet, B. K.; Taunton, J. *Nat. Chem. Biol.* **2014**, *10*, 1066−1072.

(3) (a) Chu, W. C.; Hon, C.-Y.; Danyluk, Q.; Chua, P. P. S.; Astrakianakis, G. *J. Oncol. Pharm. Pract.* **2012**, *18*, 46−51. (b) Hon, C.-Y.; Teschke, K.; Chu, W.; Demers, P.; Venners, S. *J. Occup. Environ. Hyg.* **2013**, *10*, 374−383.

(4) Hon, C. Y.; Teschke, K.; Demers, P. A.; Venners, S. *Ann. Occup. Hyg.* **2014**, *58*, 761−770.

(5) (a) Jochimsen, P. R.; Corder, M. P.; Lachenbruch, A. P.; Spaight, M. E. *Med. Toxicol.* **1988**, *3*, 59−63. (b) Fransman, W.; Roeleveld, N.; Peelen, S.; de Kort, W.; Kromhout, H.; Heederik, D. *Epidemiology* **2007**, *18*, 112−119. (c) Buschini, A.; Villarini, M.; Feretti, D.; Mussi, F.; Dominici, L.; Zerbini, I.; Moretti, M.; Ceretti, E.; Bonfiglioli, R.; Carrieri, M.; Gelatti, U.; Rossi, C.; Monarca, S.; Poli, P. *Occup. Environ. Med.* **2013**, *70*, 789−794. (d) Sessink, P. J. M.; Verplanke, A. J. W.; Herber, R. F. M.; Bos, R. P. *Int. Arch. Occup. Environ. Health* **1997**, *69*, 215−218. (e) Bussieres, J. F.; Tanguay, C.; Touzin, K.; Langlois, E.; Lefebvre, M. *Can. J. Hosp. Pharm.* **2012**, *65*, 428−435. (f) Caciara, T.; Casale, T.; Tomei, F.; Samperi, I.; Tomei, G.; Capozzella, A.; Ripamonti, K.; Scala, B.; Andreozzi, G.; Nardone, N.; Schifano, M. P.; Rosati, M. V. *Clin. Ter.* **2012**, *163*, 387−392.

(6) Fransman, W.; Huizer, D.; Tuerk, J.; Kromhout, H. *Int. Arch. Occup. Environ. Health* **2007**, *80*, 396−403.

(7) (a) Tal, S.; Salman, H.; Abraham, Y.; Botoshansky, M.; Eichen, Y. *Chem. - Eur. J.* **2006**, *12*, 4858−4864. (b) Heller, D. A.; Jin, H.; Martinez, B. M.; Patel, D.; Miller, B. M.; Yeung, T.-K.; Jena, P. V.; Hobartner, C.; Ha, T.; Silverman, S. K.; Strano, M. S. *Nat. Nanotechnol.* **2009**, *4*, 114−120. (c) Dale, T. J.; Rebek, J. *J. Am. Chem. Soc.* **2006**, *128*, 4500−4501. (d) Lee, J.-J.; Stanger, K. J.; Noll, B. C.; Gonzalez, C.; Marquez, M.; Smith, B. D. *J. Am. Chem. Soc.* **2005**, *127*, 4184−4185. (e) Gannot, Y.; Hertzog-Ronen, C.; Tessler, N.; Eichen, Y. *Adv. Funct. Mater.* **2010**, *20*, 105−110. (f) Zhang, S.-W.; Swager, T. M. *J. Am. Chem. Soc.* **2003**, *125*, 3420−3421.

(8) Dierickx, K.; Journe, F.; Gerbaux, P.; Morandini, R.; Kauffmann, J.; Ghanem, G. *Talanta* **2009**, *77*, 1370−1375.

(9) Epstein, J.; Rosenthal, R. W.; Ess, R. *Anal. Chem.* **1955**, *27*, 1435−1439.

(10) (a) Vogel, E. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **1994**, *305*, 13−32. (b) Spears, C. P. *Mol. Pharmacol.* **1981**, *19*, 496−504.

(11) Thaens, D.; Heinzelmann, D.; Böhme, A.; Paschke, A.; Schüürmann, G. *Chem. Res. Toxicol.* **2012**, *25*, 2092−2102.

(12) Gómez-Bombarelli, R.; González-Pérez, M.; Calle, E.; Casado, J. *Chem. Res. Toxicol.* **2012**, *25*, 1176−1191.

(13) (a) Maccubbin, A. E.; Caballes, L.; Riordan, J. M.; Huang, D. H.; Gurtoo, H. L. *Cancer Res.* **1991**, *51*, 886−892. (b) Johnson, L. A.; Malayappan, B.; Tretyakova, N.; Campbell, C.; MacMillan, M. L.; Wagner, J. E.; Jacobson, P. A. *Pediatr. Blood Cancer* **2012**, *58*, 708−714.

(14) (a) Loveless, A. *Nature* **1969**, *223*, 206−207. (b) Cai, Y.; Wu, M. H.; Ludeman, S. M.; Grdina, D. J.; Dolan, M. E. *Cancer Res.* **1999**, *59*, 3059−3063. (c) Williams, M. L.; Wainer, I. W. *Curr. Pharm. Des.* **1999**, *5*, 665−672.

(15) Kotali, A.; Tsoungas, P. G. *Tetrahedron Lett.* **1987**, *28*, 4321−4322.

(16) Diez-Cecilia, E.; Kelly, B.; Rozas, I. *Tetrahedron Lett.* **2011**, *52*, 6702−6704.

(17) (a) Smith, G.; Wermuth, U. D. *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2013**, *69*, 206. (b) Taher, D.; Awwadi, F. F.; Kailani, M. H. *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2013**, *69*, 1164. (c) Smith, G.; Wermuth, U. D.; Williams, M. L. *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2011**, *67*, 359. (d) Smith, G.; Wermuth, U. D. *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2010**, *66*, 1173.

(18) Silvestre, S. M.; Salvador, J. A. R. *Tetrahedron* **2007**, *63*, 2439−2445.

(19) (a) Connor, T. H.; Anderson, R. W.; Sessink, P. J.; Broadfield, L.; Power, L. A. *Am. J. Health Syst. Pharm.* **1999**, *56*, 1427−1432. (b) McDevitt, J. J.; Lees, P. S.; McDiarmid, M. A. *J. Occup. Med.* **1993**, *35*, 57−60. (c) Sessink, P. J.; Boer, K. A.; Scheefhals, A. P.; Anzion, R. B. M.; Bos, R. P. *Pharm. World Sci.* **2003**, 16−22.

(20) Christian, R. A.; Chaffee, S. K.; Hovick, C. J.; Steele, W. J. *Life Sci.* **1980**, *27*, 2595−2599.

(21) Occupational Health Guideline for Methyl Iodide. US Department of Labor. 1978.

DOI: 10.1021/acs.joc.5b01584
J. Org. Chem. 2015, 80, 9603−9609