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Dihalogen and Solvent-Free Preparation of *syn*-Bimane

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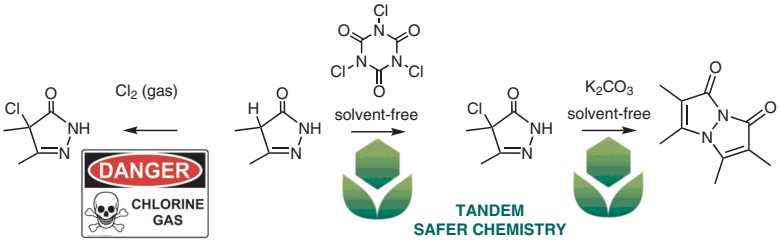

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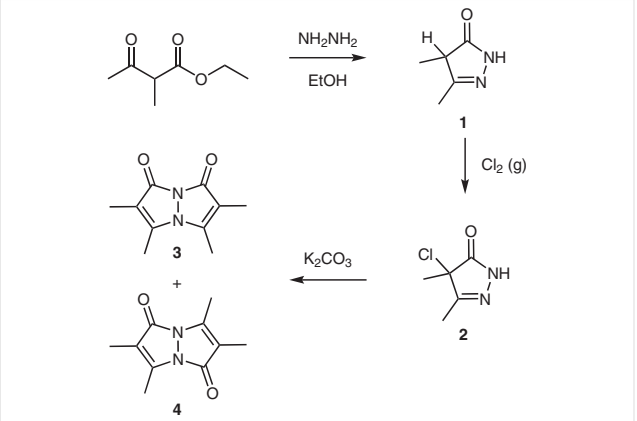
Abstract Fluorescent bimanes are low molecular weight and low toxicity molecules with applications ranging from biology to LASER dyes. The widespread use of these molecular probes has presumably been stalled by the hazards involved in their current synthetic preparation which involve handling of dangerous halogens like chlorine (gas) and bromine (liq.). The accessibility achieved by the simple and safe dihalogen and solvent-free methodologies described here open the floodgates to additional future practical applications of bimanes.

Key words bimane, fluorescent, monobromo-bimane, molecular economy, easy and safe synthesis

The first high-yielding synthetic preparation of the fluorescent *syn*-9,10-dioxabimanes was achieved by Prof. E. Kosower and co-workers about four decades ago.[1,2] This procedure has been the only one in use until today. Since their inception, bimane derivatives have found numerous applications ranging from biological tags to laser dyes.[3–7] Bimane's high quantum yield combined with its low molecular weight as well as its relatively low toxicity represent some of its advantages as biolabels over other currently more popular fluorescent probes. Recently, we showed that a *syn*-bimane adduct of α-aminobutyric acid can cross a rodent's blood-brain barrier *in vivo*.[8] In a methanol solution of *syn*-(Me,Me)bimane (**3**), for instance, absorbs light at λ_{ex} = 356 nm and emits at λ_{em} = 458 nm (in MeOH). Upon chalcogen-donor complexation with Pd(II) the fluorescence of this fluorophore can be repressed.[9] Unfortunately, the huge potential for the development of practical applications of *syn*-bimane derivatives has been hindered by the hazards involved in the single currently available synthetic procedure.[2] The crucial step in the construction of the bimane scaffold is reportedly very simple. A given chloropyrazolinone reacts in the presence of solid hydrated potassium carbonate to afford a mixture of the bicyclic *anti*- and *syn*-bimanes. The thermodynamically more stable *anti* isomer **4** is considered nonfluorescent. In contrast, the desired *syn*-bimane **3** is a highly fluorescent material that can be isolated in good yield using a combination of column chromatography and crystallization steps. Unfortunately, the preparation of the key chloropyrazolinone intermediate **2** is somehow hazardous and technically nontrivial. Following Carpino's procedure a solution of a pyrazolinone **1**, obtained from the reaction of a β-keto ester and hydrazine, in DCM or 1,2-dichloroethane is chlorinated by chlorine (gas) which can be generated by slowly pouring HCl (C) onto $KMnO_4$ or used from a chlorine tank kept in the hood (Scheme 1).[10]

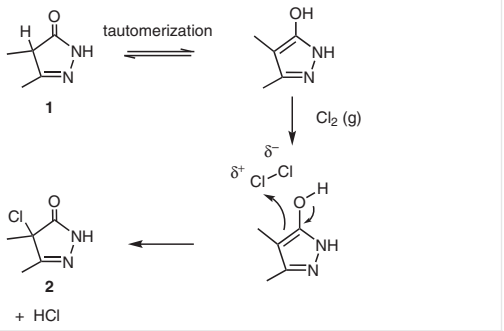


Scheme 1 Kosower's synthesis of bimane

According to the Canadian Center for Occupational Health and Safety, chlorine gas is a green-yellow gas with pungent odor. It may explode in the presence of oxidizable materials. It may cause or intensify fire. Chlorine is highly reactive, therefore incompatible with many common chemicals. Chlorine is very toxic, fatal if inhaled. This halogen is

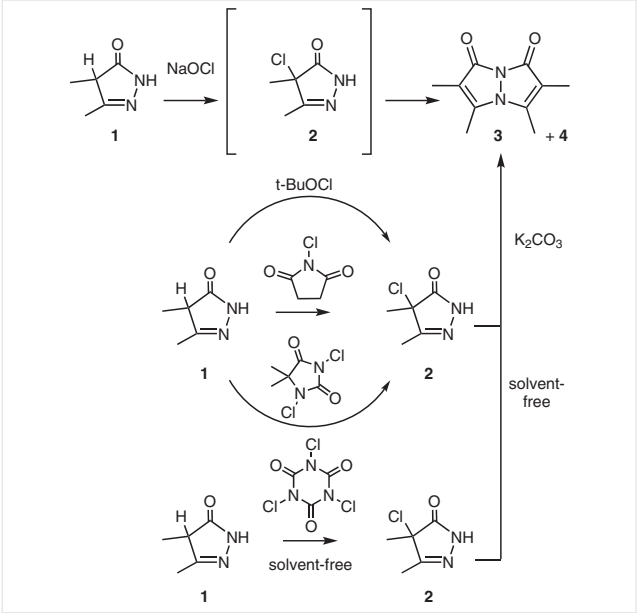
corrosive to the respiratory tract. A severe, short-term exposure may cause long-term respiratory effects. Chlorine causes severe skin burns and eye damage.[11] Definitely not suitable for the faint-of-heart. Chlorine gas should be handled with extreme care making this chlorination step cumbersome specially for nonexpert organic chemists and justifies the relatively steep price of *syn*-(Me,Me)bimane (**3**) and its derivatives when purchased from commercial sources.

Presumably, the chlorination step occurs at the carbon α to the carbonyl of **1** after tautomerization followed by nucleophilic attack at the polarizing diatomic chlorine molecule (Scheme 2).



Scheme 2 Hazardous key chlorination of pyrazolinone

We reasoned that other chlorenium (Cl^+) sources, besides the hazardous Cl_2 molecule, may also react in the same fashion in addition or instead to the expected *N*-chlorination, affording the desired chloropyrazolinone **2** intermediate.[12] The first, and perhaps most trivial, source of Cl^+ that comes to mind is NaOCl which is commonly found as a circa 5% water basic solution (pH 12.6) in the form of bleach for household use. Naively, the first thing we attempted was the chlorination reaction of **1** using lemon-scented 'Solo Bleach' brand name which was found in the cleaning room closest to the lab. To our delight, a yellowish product was readily observed and could be isolated after stirring the reaction solution at room temperature for 72 h. The isolated product was not the initially expected intermediate **2** but the fluorescent bicyclic **3**, which was generated under the existing basic reaction conditions in relatively low yield. Reportedly, the highest yields of fluorescent **3** can be obtained using the heterogeneous K_2CO_3·1.5 H_2O in an organic solvent.[2] Encouraged by this positive result we decided to explore the reaction of **1** with a different hypochlorite derivative. *tert*-Butyl hypochlorite is soluble in organic solvents and can be easily prepared by reacting *tert*-butyl alcohol with NaOCl.[13] Thus, *tert*-butyl hypochlorite was added dropwise to a solution of **1** in CCl_4 at 0 °C under an N_2 atmosphere and then stirred at room temperature for 8 h to afford the desired intermediate **2** in high yield.[14] Three additional chlorenium sources, namely *N*-chlorosuccinimide (NCS),[15] 1,3-dichloro-5,5-dimethylhydantoin (DCDMH),[16] and trichloroisocyanuric acid (TCCA)[17] were also explored in an attempt to find an effective and easy-to-handle solid reagent for the chlorination of **1**. In all cases the solid chlorination agent was added to a DCM (or CCl_4) solution of **1** at 0 °C and stirred for about 8–12 h at room temperature. After removal of the solvent, the CCl_4 insoluble succinimide, hydantoin, or cyanuric acid, respectively, were filtered out. All the reactions afforded the desired product **2** in about 80% yield without the need for further purification. All these procedures are safe and extremely easy to perform. The use of trichlorocyanuric acid as chlorinating agent is favored by us due to the high yield and intrinsic atom economy[18] of the reagent (Scheme 3) which allows the use of just 1/3 equivalents. At this stage, instead of isolating compound **2** from the reaction mixture, it is possible to add K_2CO_3·1.5 H_2O to it and isolate the desired fluorescent compound **3** in about 75% yield (and about 20% **4**) in a convenient one-pot process. The scalability of this process was demonstrated by starting the reaction from 0.5 g and 2 g of **1** with similar overall yield. The yield of our tandem procedure is significantly higher than the reported two consecutive steps[2] (50.3% overall yield) and it has become our preferred route to prepare bimanes.



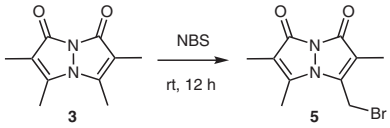
Scheme 3 Safe and easy access to the key chloropyrazolinone intermediate **2** and bimanes using either sodium hypochlorite, *tert*-butyl hypochlorite, NCS, DCDMH, and TCCA

Organic solvents represent most of the waste produced by regular synthetic procedures. The use of benign solvents like water or no solvent at all, even at the expense of a slightly lower yield, is a central premise while trying to achieve environmentally responsible chemical processes. In this case, grinding **1** (previously heated over its reported melting point of 56 °C) and solid trichlorocyanuric acid at

room temperature, in the absence of any additional solvent, for 15 min affords the desired product in 69% yield (the same yield was obtained after grinding for 30 min).[19] The fluorescent bicyclic product **3** can then be prepared as reported, via the reaction of **2** in the presence of K_2CO_3·1.5 H_2O in an heterogeneous system.[2]

Since the chloropyrazolinone intermediate is a liquid at room temperature we decided to attempt this step in the absence of any additional solvent. Indeed, the solvent-free formation of the bicyclic bimane scaffold proceeds smoothly affording *syn*-bimane in 73% yield.[20] This yield is comparable to that reported in the literature. The solvent-free procedure is very convenient, this reaction ends in less than 5 min and, in contrast to the previously reported procedure in solution, the resulting mixture of bimanes can be easily separated from K_2CO_3 simply by solubilization of the bicyclic compounds in DCM. These two solvent-free steps can be carried out in tandem by just adding solid potassium carbonate to the same mortar where liquid **2** is obtained, without isolation of the intermediate. Following this 'green' approach, **3** can be isolated in only 38% overall yield for the combined two steps. It must be noted that all the described solvent-free reactions are exothermic and should be performed in a ventilated hood with care.

To take advantage of bimane **3** as a fluorescent probe it is necessary to convert into an alkylating agent such as monobromo bimane **5**. Conveniently, the fluorescence of **5** is very weak but most of it is recovered when it reacts with thiol nucleophiles.[21] Reportedly, the bromination of a β-alkyl H of **3** can be achieved via the reaction of **3** with Br_2 (liq.).[2] Handling liquid bromine may be considered less dangerous than working with chlorine gas but in order to make the whole reaction sequence safer and effective, we decided to replace the use of liquid bromine by solid *N*-bromosuccinimide (NBS, Scheme 4).[22] The reaction proceeds smoothly at room temperature over a period of 12 h affording the desired **5** in yields comparable to the obtained by the original bromination procedure.



Scheme 4 Use of *N*-bromosuccinimide in the modified preparation of monobromo bimane

In summary, we have shown that fluorescent *syn*-(Me,Me)bimane and its reactive bromo derivative can be prepared using our very simple-and-safe procedures.[23] The methodology presented herein circumvents the need of direct handling of hazardous chlorine (gas) and bromine (liq.), it draws closer to the concepts of 'green chemistry' and can be performed successfully by any scientist with basic knowledge and experience in organic chemistry practices. We hope this significant improvement will contribute to uplift the bimane family of fluorophores to a more preponderant place within the arsenal of available fluorescent probes, where it belongs.

Acknowledgment

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(14) **Synthesis of 3,4-Dimethyl-4-chloro-2-pyrazolin-5-one (2) by Chlorination of 3,4-Dimethyl-2-pyrazolin-5-one (1) Using *tert*-Butyl Hypochlorite**
3,4-Dimethyl-2-pyrazoline-5-one (**1**, 0.49 g, 4.37 mmol) was dissolved in 8 mL of CCl_4 under a nitrogen atmosphere. The solution was cooled to 0 °C. *tert*-Butyl hypochlorite (0.49 mL, 4.37 mmol, CAS 507-40-4) was slowly added to the reaction mixture dropwise. The reaction mixture was stirred at rt. After stirring for 8 h, the solvent was removed using under reduced pressure at 40 °C, yielding 0.60 g the product **2** (93% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.68 (s, 3 H), 2.12 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 12.6, 21.7, 60.0, 159.9, 173.8 ppm. ESI-MS: *m/z* calcd for $C_5H_8ClN_2O$: 147.0325 [MH^+]; found: 147.0352.
(15) **Synthesis of 3,4-Dimethyl-4-chloro-2-pyrazolin-5-one (2) by Chlorination of 3,4-Dimethyl-2-pyrazolin-5-one (1) Using *N*-Chlorosuccinimide**
3,4-Dimethyl-2-pyrazoline-5-one (**1**, 0.5 g, 4.45 mmol) was dissolved in 10 mL of DCM. The solution was cooled to 0 °C. NCS

(0.59 g, 4.45 mmol, CAS 128-09-6) was added to the reaction mixture over a period of 30 min. The reaction mixture was stirred at rt. After stirring for 12 h. The solvent was removed using under reduced pressure at 40 °C. The resulting residue was dissolved in CCl_4, and filtered to remove solid succinimide and yielding a filtrate. The solvent was removed from the filtrate yielding 0.52 g the product **2** (80% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.68 (s, 3 H), 2.12 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 12.6, 21.7, 60.0, 159.9, 173.8 ppm.

(16) **Synthesis of 3,4-Dimethyl-4-chloro-2-pyrazolin-5-one (2) by Chlorination of 3,4-Dimethyl-2-pyrazolin-5-one (1) Using 1,3-Dichloro-5,5-dimethylhydantoin**
3,4-Dimethyl-2-pyrazoline-5-one (**1**, 0.5 g, 4.45 mmol) was dissolved in 10 mL of CCl_4. The solution was cooled to 0 °C. 1,3-Dichloro-5,5-dimethylhydantoin (0.44 g, 2.22 mmol, CAS 118-52-5) was slowly added to the reaction mixture over a period of 30 min. The reaction mixture was stirred at rt. After stirring for 12 h, a solid precipitate was observed. The precipitate was removed by filtration. The solvent was removed under reduced pressure at 40 °C, yielding 0.50 g the product **2** (76% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.68 (s, 3 H), 2.12 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 12.6, 21.7, 60.0, 159.8, 173.9 ppm.

(17) **Synthesis of 3,4-Dimethyl-4-chloro-2-pyrazolin-5-one (2) by Chlorination of 3,4-Dimethyl-2-pyrazolin-5-one (1) Using Trichloroisocyanuric Acid**
3,4-Dimethyl-2-pyrazoline-5-one (**1**, 0.5 g, 4.45 mmol) was dissolved in 10 mL of DCM. The solution was cooled to 0 °C. Trichloroisocyanuric acid (0.34 g, 1.48 mmol, CAS 87-90-1) was slowly added to the reaction mixture over a period of 30 min. The reaction mixture was stirred at rt. After stirring for 12 h, a solid precipitate of cyanuric acid was observed. The cyanuric acid precipitate was removed by filtration. The solvent was removed under reduced pressure at 40 °C, yielding 0.54 g the product **2** (82% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.68 (s, 3 H), 2.12 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 12.6, 21.7, 60.0, 159.9, 173.8 ppm.

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(19) **Solvent-Free Synthesis of 3,4-Dimethyl-4-chloro-2-pyrazolin-5-one (2) by Chlorination of 3,4-Dimethyl-2-pyrazolin-5-one (1) Using Trichloroisocyanuric Acid**
3,4-Dimethyl-2-pyrazoline-5-one (**1**, 0.2 g, 1.78 mmol) was finely ground by mortar and pestle. Trichloroisocyanuric acid (0.14 g, 0.59 mmol, CAS 87-90-1) was added to the fine powder and grinding was continued. The progress of the reaction was monitored by TLC. The reaction reached its completion after 15 min. Then, DCM was added to the reaction mixture. The insoluble cyanuric acid was removed by filtration. The solvent was removed under reduced pressure at 40 °C, yielding 0.18 g 3,4-dimethyl-4-chloro-2-pyrazolin-5-one (**2**, 69% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.68 (s, 3 H), 2,12 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 12.6, 21.7, 60.0, 159.8, 173.9 ppm. ESI-MS: *m/z* calcd for $C_5H_8ClN_2O$: 147.0325 [MH^+]; found: 147.0352.

(20) **Solvent-Free Synthesis of 9,10-Dioxa-*syn*-(Me,Me)bimane (3)**
3,4-Dimethyl-4-chloro-2-pyrazolin-5-one (0.2 g, 1.36 mmol) was melted and added to a scintillation vial containing K_2CO_3·1.5 H_2O (0.945 mg, 5.71 mmol). The two components were rapidly mixed with a spatula at rt. The progress of the reaction was monitored by TLC. The reaction reached completion after 15 min. DCM was added to the reaction mixture in five portions and the combined organic extract was evaporated under reduced pressure. The reaction mixture was purified by column chromatography eluting with hexane/ethyl acetate (1:3). The desired 9,10-dioxa-*syn*-(Me,Me)bimane (**3**) was isolated (0.096 g, 73% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.80 (s, 3 H), 2.29 (s, 3 H) ppm. ESI-MS: *m/z* calcd for $C_{10}H_{13}N_2O_2$: 193.0977 [MH^+]; found: 193.0992.

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(22) **Synthesis of Monobromobimane (5) by Bromination of *syn*-(Me,Me)bimane (3) Using NBS**
syn-(Me,Me)bimane (**3**, 0.5 g, 2.6 mmol) was dissolved in 50 mL acetonitrile. The reaction mixture was cooled to 0 °C. Recrystallized NBS (0.46 g, 2.6 mmol, CAS. 128-08-5) was slowly (pinchwise) added to the cooled reaction mixture over a period of 30 min. After the addition of *N*-bromosuccinimide was complete, the reaction mixture was stirred at rt for 12 h. The solvent was then removed under reduced pressure. The residue was purified using flash chromatography eluted with hexane/ethyl acetate (1:1). The product **5** (0.35 g, 50% yield) was isolated. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.84 (s, 3 H), 1.89 (s, 3 H), 2.45 (s, 3 H), 4.32 (s, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 6.9, 11.5, 17.8, 113.2, 115.4, 144.2, 146.0, 159.8, 160.7 ppm. ESI-MS: *m/z* calcd for $C_{10}H_{12}BrN_2O_2$: 271.0082 [MH^+]; found: 271.0067.

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