Caged compounds have been introduced to biological studies with the synthesis of caged ATP by Kaplan et al. in 1978.\(^1\) Since then, a variety of biologically relevant molecules have been caged and used to elucidate cellular processes.\(^2,3\) These molecules include small-molecule ligands for proteins,\(^4\) fluorescent probes,\(^5\) calcium chelators,\(^6\) oligonucleotides,\(^7\) peptides,\(^8\) and proteins.\(^9\) In order to generate caged compounds, a photolabile protecting group, most often a 2-nitrobenzyl group, is attached to an oxygen, sulfur, or nitrogen atom of a biologically active molecule thereby inactivating its function (1 in Scheme 1). Upon exposure of the caged compound to UV light, a photochemical reaction is initiated, generating the intact biologically active molecule 2, typically on a millisecond timescale. Photolysis produces a by-product as well; namely, the nitrosobenzene 3. The caging group should exhibit a number of characteristics,\(^2\) including: 1) generating a functionally inactive (bio)molecule; 2) rapid photolysis upon exposure to UV light that is not damaging to a biological system (>360 nm); and 3) stability under physiological conditions, i.e., resistance to degrading enzymes and to hydrolysis at pH 7.4.

In the course of our studies to develop light-controllable means to regulate biological processes, we caged the N-heterocyclic small organic molecule theophylline.\(^10\) There, the NVOC (6-nitroveratryl chloroformate) caging group, which is a classical caging group for nitrogen centers, comprised of a carbamate linkage between a nitrobenzyl alcohol and the nitrogen atom, did not lead to a stably caged molecule. A caged theophylline molecule, stable under aqueous conditions was obtained by direct alkylation with an ortho-nitrobenzyl group. However, prolonged decaging times have been observed. We solved this problem by installing an oxymethyl unit between the nitrogen atom and the benzyl group, generating an NPOM (6-nitropiperyloxy)methyl amino caging group.

Hence, it seemed that the classical NVOC caging of amines may not be generally applicable to aromatic N-heterocyclic structures, which prompted us to explore the NPOM caging group for this purpose. A set of aromatic N-heterocycles with biological relevance has been selected, including benzimidazole (4\(a\)), (protected) histidine (5\(a\)), purine (6\(a\)), benzotriazole (7\(a\)), and indazole (8\(a\)) (Figure 1). These molecules represent core structures found in a variety of biologically active molecules, secondary metabolites, amino acids, and nucleic acids.\(^11\) Photochemical regulation of the activity of these molecules would greatly facilitate studies of the biological processes in which they are involved. Moreover, the NPOM caging group has potential in synthetic applications since it is stable under a variety of conditions and can be cleaved in a fashion orthogonal to most amino protecting groups.\(^12\)

![Scheme 1](image-url)  
**Scheme 1** Generalized schematic of a decaging reaction. Application of an ortho-nitrobenzyl caged compound 1 in the decaging reaction towards 2. \(X = O,\text{NH, OC(O)}\text{NH, or S; } R, R’ = \text{OMe or OCH}_2\text{O; } R” = \text{H, Me, or CO}_2\text{H.}\)

![Figure 1](image-url)  
**Figure 1** Nitrogen containing heterocycles used in this study.

---

**Abstract:** Biologically relevant nitrogen-containing heterocycles were photo-protected using an NPOM caging group that is stable under physiological conditions but readily decages under irradiation with non-photodamaging UV light.

**Key words:** bioorganic chemistry, heterocycles, photocaging, protecting groups
To prove our hypothesis that the traditional NVOC group is not a suitable caging group for 4a–8a, we examined the stability of the ethylcarbamates 4c–8c (data not shown). As suspected, none of the carbamates 4c–8c were stable under aqueous conditions (Tris buffer/MeOH, pH 7.4), and hydrolysis to 4a–8a was complete in 1–12 hours as determined by LC/MS analysis, thus rendering the use of an NVOC caging group impractical for these molecules. This instability is most likely due to the leaving group an NVOC caging group impractical for these molecules.

As suspected, none of the carbamates were perfectly stable in an aqueous environment (H2O–MeOH = 7:3). UV spectra of all caged molecules show an UV light of 365 nm. Decaging experiments were conducted in MeOH (0.1 mM) using a hand-held UV lamp (365 nm, 25 W, 15 min). Yields were determined by LC/MS analysis.

In summary, a new caging group for N-heterocyclic molecules has been developed. The caged molecules are stable under physiological conditions and decage rapidly and quantitatively under irradiation with non-photodamaging UV light.

All solvents and reagents were purchased from Aldrich or Fisher. Chromatography was carried out on silica gel, Merck, 60Å. NMR spectra were obtained on a Varian 300 MHz spectrometer. Decagings were conducted with a handheld Spectroline UV lamp (25 W) at 365 nm. Absorbance spectra were obtained on an HP UV/Vis Spectrometer 8453. LC/MS analysis was performed on an Agilent 1100 MSD system with a ZorbaxSB C-18, 3.5 μm pore size 4.5 × 100 mm column. HRMS data was obtained using a JEOL HX110HF (accelerating voltage 10 keV, 40 °C ion source temperature).

Caging for 4a–5a and 7a–8a; General Procedure
NaH (1.2 equiv, 60% suspension in mineral oil) was added to a solution of the heterocycle (50 mg) in THF or DMF (1 mL), and the mixture was stirred at r.t. for 1 h. The solution was cooled to 0 °C and NaH (1.5 equiv) dissolved in THF or DMF (1 mL) was added dropwise. The mixture was stirred at 0 °C for 12 h, and H2O (5 mL) was added. The layers were separated and the aqueous layer was extracted with CH2Cl2 (3 × 5 mL). The organic layers were combined, dried (MgSO4), filtered, and the solvent was evaporated in vacuo. The product was purified by silica gel chromatography.

1-[(1-(6-Nitro-1,3-benzodioxol-5-yl)ethoxy)methyl]-1H-benzimidazole (4b)
According to the general procedure, benzimidazole (50 mg, 0.42 mmol) was caged using NaH (12 mg, 0.50 mmol) and NaCl (164 mg, 0.63 mmol) in DMF. The product was flash-purified using an EtOAc–hexanes (60:40) mixture with 1% Et3N as the eluent, affording 4b (119 mg) as a white solid; yield: 83%; Rf = 0.25 (hexanes–EtOAc, 2:1).

Caging for 6b–8b and 7d–8d

Table 1  Heterocycle Caging and Decaging Results

<table>
<thead>
<tr>
<th>Caged compound</th>
<th>Caging (%)</th>
<th>Decaged compound</th>
<th>Decaging (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4b</td>
<td>83</td>
<td>4a</td>
<td>87</td>
</tr>
<tr>
<td>5b</td>
<td>72</td>
<td>5a</td>
<td>97</td>
</tr>
<tr>
<td>6b</td>
<td>78</td>
<td>6a</td>
<td>100</td>
</tr>
<tr>
<td>7b</td>
<td>87</td>
<td>7a</td>
<td>83</td>
</tr>
<tr>
<td>7d</td>
<td>7a</td>
<td>7a</td>
<td>93</td>
</tr>
<tr>
<td>8b</td>
<td>84</td>
<td>8a</td>
<td>99</td>
</tr>
<tr>
<td>8d</td>
<td>8a</td>
<td>8a</td>
<td>92</td>
</tr>
</tbody>
</table>

*Decaging was conducted in MeOH (0.1 mM) using a handheld UV lamp (365 nm, 25 W, 15 min). Yields were determined by LC/MS analysis.

Scheme 2  Preparation of NPOMCl (11) and its application in the synthesis of caged heterocycles 4b–8b.

The NPOM caging group was installed by reacting the oxymethylene chloride 11 with the corresponding heterocyclic molecules 4a–8a using NaH or KH as the base and THF or DMF as the solvent (Scheme 2). All reactions proceeded in good to excellent yield (Table 1). In the case of benzo[4,5]thieno[3,2-b]thiophene (7a) and indazole (8a), two regioisomeric alklylation products (7b and 7d, and 8b and 8d) were obtained. This is in agreement with previous observations described in the literature. 18 Only in the formation of 6b we were able to suppress the formation of regioisomers. As expected, all caged heterocycles 4b–8b and 7d–8d were perfectly stable in an aqueous environment (H2O–MeOH = 7:3). UV spectra of all caged molecules show an absorbance maximum between 336 nm and 345 nm, making them susceptible to decaging with non-photodamaging UV light of 365 nm. Decaging experiments were conducted with 4b–8b and 7d–8d in MeOH (0.1 mM) at room temperature. The solutions were irradiated with UV light (handheld UV lamp, 25 W, 365 nm) for 15 min. Decaging reactions were analyzed by LC/MS indicating the formation of 4a–8a in 83–100% (Table 1).
UV (CH3CN): $\lambda_{\text{max}}$ (e) = 247 (22756), 275 (11986), 283 (9577), 339 nm (5394).

HRMS-FAB: m/z calecd for C$_{17}$H$_{15}$N$_{3}$O$_{3}$ [M + H]$^+$: 342.1090; found: 342.1096.

tert-Butyl 1-(Methoxycarbonyl)-2-[[1-(6-nitro-1,3-benzodioxol-5-yl)ethoxy]methyl]-1H-imidazol-4-yl]ethylcarbamate (5b)

According to the general procedure, Boc-L-histidine methyl ester (50 mg, 0.18 mmol) was caged using NaH (5 mg, 0.20 mmol) and 11 (75 mg, 0.29 mmol) in THF. The product was flash-purified using an EtOAc–hexanes (75:25) mixture with 1% Et$_3$N as the eluent, affording 5b (65 mg) as a yellow solid; yield: 72%; $R_f$ = 0.5 (EtOAc).

$^1$H NMR (CDCl$_3$): $\delta$ = 1.44 (m, 12 H, CH$_2$, t-C$_6$H$_5$), 2.96 (m, 2 H, CH$_2$), 3.69 (s, 3 H, OCH$_3$), 4.49 (br s, 1 H, CH), 5.12 (m, 3 H, NCH$_2$O), 5.80 (m, 1 H, NH), 6.10 (s, 1 H, OCH$_2$O), 6.13 (s, 1 H, OCH$_2$O), 6.68 (m, 1 H, ArH), 7.02 (s, 1 H, ArH), 7.33 (s, 1 H, ArH), 7.41 (s, 1 H, ArH).

$^{13}$C NMR (CDCl$_3$): $\delta$ = 133.6, 135.5, 136.0, 138.3, 139.3, 140.9, 142.6, 143.7, 144.8, 146.9, 150.7.

HRMS-FAB: m/z calecd for C$_{21}$H$_{23}$N$_{3}$O$_{5}$ [M + H]$^+$: 493.1935; found: 493.1915.

7-[[1-(6-Nitro-1,3-benzodioxol-5-yl)ethoxy]methyl]-7H-purine (6b)

To a solution of purine (50 mg, 0.42 mmol) in DMF (1 mL) was added KH (20 mg, 0.50 mmol), and 18-crown-6 (6.5 mg, 0.025 mmol), and the solution was left to stir at r.t. for 1 h. It was then cooled to −78 °C and 11 (75 mg, 0.29 mmol) in THF was added. The reaction was allowed to proceed for 12 h while it warmed up to 0 °C. H$_2$O (5 mL) was added and the cooled to −78 °C and added KH (20 mg, 0.50 mmol), and 18-crown-6 (6.5 mg, 0.025 mmol) was added dropwise. The reaction was then allowed to proceed for 12 h while it warmed up to 0 °C. H$_2$O (5 mL) was added and the mixture was extracted with CH$_2$Cl$_2$ (3 × 5 mL). The organic layers were combined, dried (MgSO$_4$), filtered, and the solvent was evaporated in vacuo. The product was purified by silica gel chromatography using an EtOAc–hexanes mixture (80:20) with 1% Et$_3$N as the eluent, affording 6b (112 mg) as a white solid; yield: 78%; $R_f$ = 0.4 (EtOAc–hexanes, 3:1).

$^1$H NMR (CDCl$_3$): $\delta$ = 1.51 (d, J = 6.3 Hz, 3 H, CH$_3$), 5.33 (q, J = 6, 1 H, CH), 5.58 (s, 1 H, OCH$_2$O), 5.59 (s, 1 H, OCH$_2$O), 6.06 (s, 2 H, NCH$_2$O), 6.99 (s, 1 H, ArH), 7.31 (s, 1 H, ArH), 8.13 (s, 1 H, ArH), 8.90 (s, 1 H, ArH), 9.07 (s, 1 H, ArH).

$^{13}$C NMR (CDCl$_3$): $\delta$ = 124.2, 126.4, 127.3, 127.9, 128.6, 129.1, 131.6, 132.2, 133.5, 142.5, 144.7, 148.1, 151.6, 152.2, 153.3.

UV (CH$_2$Cl$_2$): $\lambda_{\text{max}}$ (e) = 247 (24408), 338 nm (6619).

HRMS-FAB: m/z calecd for C$_{17}$H$_{15}$N$_{3}$O$_{3}$ [M + H]$^+$: 344.0995; found: 344.1004.

1-[[1-(6-Nitro-1,3-benzodioxol-5-yl)ethoxy]methyl]-1H-1,2,3-benzotriazole (7b) and 2-[[1-(6-Nitro-1,3-benzodioxol-5-yl)ethoxy]methyl]-1H-1,2,3-benzotriazole (7d)

According to the general procedure, benzotriazole (50 mg, 0.42 mmol) was caged using NaH (12 mg, 0.50 mmol) and 11 (91 mg, 0.35 mmol) in THF. The product was flash-purified using a hexanes–EtOAc (85:15) mixture with 1% Et$_3$N as the eluent, affording 7b (89 mg, 62% yield) and 7d (36 mg, 25% yield) as white solids.

$R_f$ = 0.2 (hexanes–EtOAc, 4:1).

UV (CH$_3$Cl$_2$): $\lambda_{\text{max}}$ (e) = 248 (19975), 286 (8256), 345 nm (5947).

HRMS-FAB: m/z calecd for C$_{17}$H$_{15}$N$_{3}$O$_{3}$ [M + H]$^+$: 343.1042; found: 343.1042.

7d

$R_f$ = 0.4 (hexanes–EtOAc, 4:1).

UV (CH$_3$Cl$_2$): $\lambda_{\text{max}}$ (e) = 247 (22756), 286 (18340), 345 nm (5394).

HRMS-FAB: m/z calecd for C$_{17}$H$_{15}$N$_{3}$O$_{3}$ [M + H]$^+$: 343.1042; found: 343.1042.

1-[[1-(6-Nitro-1,3-benzodioxol-5-yl)ethoxy]methyl]-1H-indazole (8b) and 2-[[1-(6-Nitro-1,3-benzodioxol-5-yl)ethoxy]methyl]-1H-indazole (8d)

According to the general procedure, indazole (20 mg, 0.17 mmol) was caged using NaH (5 mg, 0.20 mmol) and 11 (40 mg, 0.154 mmol) in THF. The product was flash-purified using an EtOAc–hexanes (20:80) mixture with 1% Et$_3$N as the eluent, affording 8b (25 mg, 48% yield) and 8d (19 mg, 36% yield) as yellow oils.

$R_f$ = 0.33 (hexanes–EtOAc, 3:1).

UV (CH$_3$Cl$_2$): $\lambda_{\text{max}}$ (e) = 247 (10715), 283 (8672), 296 (8489), 349 nm (4246).

HRMS-FAB: m/z calecd for C$_{17}$H$_{15}$N$_{3}$O$_{3}$ [M + H]$^+$: 342.1090; found: 342.1089.

8d

$R_f$ = 0.5 (hexanes–EtOAc, 3:1).

UV (CH$_3$Cl$_2$): $\lambda_{\text{max}}$ (e) = 236, 724, 77.1, 102.8, 104.6, 106.6, 109.4, 121.2, 121.3, 125.0, 127.1, 134.9, 136.9, 140.0, 141.9, 146.7, 151.9.

UV (CH$_3$Cl$_2$): $\lambda_{\text{max}}$ (e) = 250 (14117), 288 (7314), 299 (6508), 347 nm (4791).

HRMS-FAB: m/z calecd for C$_{17}$H$_{15}$N$_{3}$O$_{3}$ [M + H]$^+$: 342.1090; found: 342.1089.
oxide over 20 min (2.96 g, 9.48 mmol). The solution was then stirred at 0 °C for 2 h. The mixture was poured into EtO (250 mL) and washed withaq 1.0 M NaOH (3 × 250 mL), brine (250 mL), dried (MgSO4) and filtered. The product was purified by silica gel chromatography using a hexanes–EtOAc mixture (85:15) affording 10 as a brown solid; yield: 435 mg (71%).

1H NMR (CDCl3): δ = 1.50 (d, J = 6.3 Hz, 3 H, CH3), 2.22 (s, 3 H, SCHO), 4.29 (d, J = 11.4 Hz, 1 H, OCH2), 4.60 (d, J = 11.4 Hz, 1 H, OCH2), 5.45 (q, J = 6.3 Hz, 1 H, CH), 6.11 (m, 2 H, CH3), 7.16 (s, 1 H, ArH), 7.46 (s, 1 H, ArH).

UV (CH2Cl2): λmax (ε) = 2456 (1264), 275 (5800), 342 nm (4166).


5-[1-(Chloromethoxy)ethyl]-6-nitro-1,3-benzodioxole (11) SO2Cl2 (29.9 mg, 179 µL, 0.22 mmol) was added to a solution of 10 (50 mg, 0.184 mmol) in CH2Cl2 (1 mL) at 0 °C, and the mixture was stirred for 4 h. The solvent and excess SO2Cl2 were evaporated in vacuo. This procedure afforded 11 as a yellow oil (quantitative conversion) which was directly used without further purification.

1H NMR (CDCl3): δ = 1.57 (d, J = 6 Hz, 3 H, CH3), 5.24 (d, J = 5.7 Hz, 1 H, OCH2), 5.52 (d, J = 5.7 Hz, 1 H, OCH2), 5.56 (q, J = 6 Hz, 1 H, CH), 6.13 (m, 2 H, OCH2), 7.08 (s, 1 H, ArH), 7.49 (s, 1 H, ArH).

13C NMR (CDCl3): δ = 23.4, 73.3, 80.5, 103.2, 105.4, 106.6, 135.7, 142.1, 147.4, 152.6.

Decaging of Derivatives 4b–8b and 7d–8d; General Procedure

Decaging of 4b–8b and 7d–8d was conducted by irradiating a 0.1 mM solution of the caged heterocycle in MeOH in a quartz cuvette for 15 min using a handheld UV lamp (25 W) at a 365 nm. The solution was then analyzed by LC/MS. Decaging yields were assessed by integration of the corresponding peak and comparison with a 0.1 mM sample of 4a–8a.

Acknowledgment

We thank Douglas D. Young for technical assistance with the LC/MS measurements. Mass spectra were obtained at the NSF and NCBC funded Mass Spectrometry Laboratory for Biotechnology. This research was supported in part by research grant no. 5-FY05-1215 from the March of Dimes Birth Defects Foundation.

References