Unexpected photolytic decomposition of alkyl azides under mild conditions†

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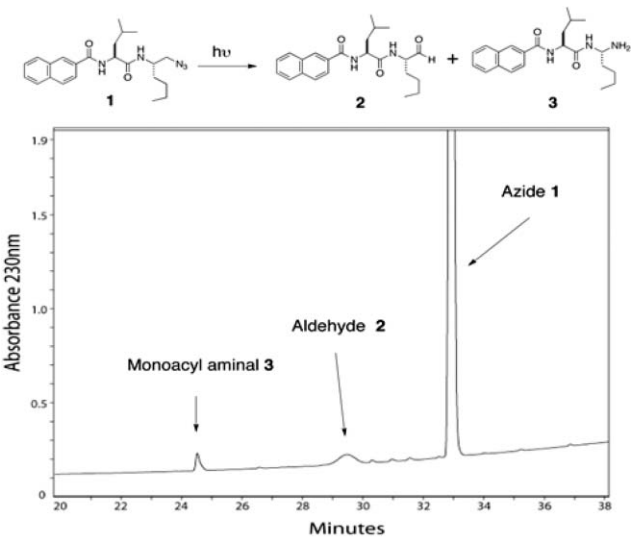
Alkyl azides have been found to undergo an unexpectedly facile photodecomposition under mild conditions (laboratory light) and the reaction gives rise to small amounts of aldehydes and monoacyl aminal rearrangement products that can dramatically impact on the uses of azides in chemistry, biology and medicine.

Organic azides are becoming established as valuable and versatile synthetic intermediates, protecting groups, photoaffinity labels and drug components.[1] Their value is highlighted by recent uses of the Huisgen reaction ('click chemistry'),[2] in cell-surface remodelling *in vivo*,[3] and to inhibit cysteine proteases.[4] We now report that peptidic alkyl azides are unexpectedly susceptible to photolytic degradation, even under mild exposure to laboratory light at room temperature, giving traces of aldehyde and monoacyl aminal products. This unexpected degree of sensitivity to light-induced decomposition has very important ramifications for the storage, chemical reactivity, and biological properties of azides.

For example, when azide 2-Nap–Leu–Nle–N_3 **1** was stored in the solid state in a clear soda-glass vial at room temperature under fluorescent laboratory light, it decomposed slowly at ~1% per day to decomposition products aldehyde **2** and monoacyl aminal **3** (Fig. 1). The significance of this result is that azide **1** has been reported to potently inhibit the proteolytic enzyme cathepsin K (IC_{50} 1 nM).[4] Yet aldehydes like **2** are extremely potent enzyme inhibitors in their own right and can compromise measurements of enzyme inhibition by azides. An impurity of <1% aldehyde 2-Nap–Leu–Nle–CHO (IC_{50} ~70 pM) could account for much of the reported[4] inhibitor potency of **1**, and Fig. 1 shows a clear correlation between aldehyde formation in the solid state and increasing inhibitor potency. In another context, impurities such as **2** and **3** have highly reactive functional groups that can potentially contaminate synthetic transformations of azides in chemical and biological applications, potentially leading to undesirable side products.

The trace decomposition products **2** and **3** were discovered after preparing large quantities of **1** (>0.1–1 g) and observing variations in enzyme inhibitor potency of aged samples. Although **1** was quite stable as a solid stored in the dark at room temperature for 20 days, it decomposed in glass vials under fluorescent laboratory light, decomposition being much faster (~0.5% h^{-1}) in water–acetonitrile solutions than in the solid state (~1% per day). The same decomposition occurred even more rapidly when solid **1** was exposed in glass to direct sunlight (>33% h^{-1}). These observations indicated unexpected chemical instability of **1** under mild exposure to light.



Time, h	Aldehyde **2** (%)	IC_{50} (catK), nM
0	0	459
6	0.24	168
14	0.73	37
34	1.27	18

Fig. 1 Analytical HPLC trace after 34 h irradiation of solid Nap–L–Nle–N_3 **1** under fluorescent laboratory lights. Decomposition was found to give aldehyde **2** (rt = 29.8 min) and monoacyl aminal **3** (rt = 24.5 min), as the major products. The formation of aldehyde **2** (assessed by rpHPLC) from solid **1** correlated with increased inhibition of cathepsin K.

To investigate the decomposition, samples of **1** were irradiated (8 W Hg lamp, 2 days). The products **2** and **3** were isolated by preparative HPLC and their identities determined by mass and NMR spectroscopy.‡ Absolute stereochemistries (Fig. 1) were established by independent synthesis of aldehyde **2** (ESI†), and of monoacyl aminal **3** by Hoffman rearrangement of the primary amide 2-Nap–L–Nle–NH_2 (ESI†). Hoffman rearrangement of peptidic primary amides is known to proceed with retention of configuration at the migrating α-carbon of the C-terminal amino acid.[7] Monoacyl aminals are known to be stable enough to isolate.[5,6] A Schmidt rearrangement can account for formation of **2** and **3** from **1** (Fig. 2),[1] but is only known to occur under pyrolysis, prolonged thermolysis or high intensity UV irradiation (450 W, 254 nm, Hg).[8,9]

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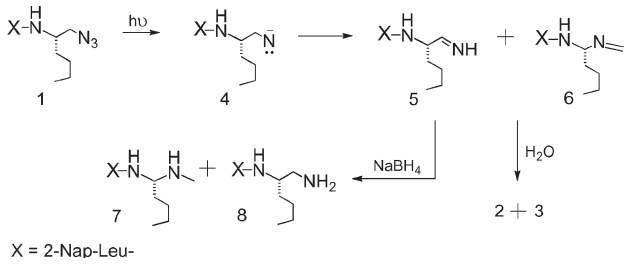

Fig. 2 Possible mechanism of azide decomposition.

Aldehyde **2** and monoacyl aminal **3** could potentially arise through hydrolysis of intermediate imines **5** and **6** (Fig. 2). The decomposition of alkyl azides like **1** to an imine can in principle occur *via* a concerted process[10] or *via* the singlet nitrene intermediate **4** (Fig. 2). The literature currently suggests that irradiation of alkyl azides does not generally involve formation of nitrenes, but rather the excited state of the azide rearranges to yield imines directly.[10,11] Indirect evidence for imines was obtained when **1** was irradiated for 3 days in water–acetonitrile solution under laboratory light in the presence of $NaBH_4$. Consistent with formation of **5** (Fig. 2), product analysis by analytical HPLC revealed primary amine **8** (25%), the primary alcohol arising from reduction of aldehyde **2** (8%), and unreacted **1** (67%). By contrast, only a trace of reduction (<1%) occurred when a solution of **1** was kept in the dark under otherwise identical conditions. Pure primary amine **8** was isolated (14% yield) from a large-scale reaction of **1** with $NaBH_4$ in sunlight and its identity was established by ^{1}H NMR and mass spectroscopy.‡ Secondary amine **7** was not detected, indicating that monoacyl aminal **3** might not be produced *via* this mechanism.

The rate of azide decomposition was also found to depend on chemical composition. When water–acetonitrile solutions of Boc–L–N_3, Ac–D–E–L–D–N_3, and 2-Nap–L–Nle–N_3 were left in clear glass vials and exposed to sunlight for 3 h, HPLC analysis showed negligible decomposition for Boc–L–N_3 and Ac–D–E–L–D–N_3 (no aldehyde detected), but 2-Nap–L–Nle–N_3 decomposed extensively (27% aldehyde). This suggests that the 2-naphthyl moiety in **1** might render the azide more susceptible to photolysis. Compound **1** is naturally fluorescent (Ex_{max} 230 nm, Em_{max} 359 nm) due principally to the presence of the 2-naphthyl group. Photodecomposition might occur *via* a resonance energy transfer process. Consistent with this hypothesis, irradiation of solid 2-Nap–V–A–D–N_3 **9** in sunlight gave extensive decomposition (Fig. 3), whereas incorporation of an internal fluorescence quencher (*p*-nitrophenylalanine) rendered 2-Nap–V–(4-NO_2)F–D–N_3 **10** stable to sunlight under identical conditions (Fig. 3).

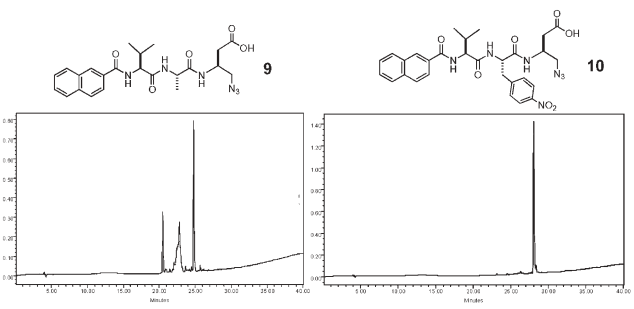

Fig. 3 HPLC chromatograms showing extensive degradation of azide **9**, but not azide **10**, after 2.5 h sunlight exposure in the solid state.

Table 1 Inhibitor potencies of 'aldehyde-free' alkyl azides **1**, **9**, **11** and **12** against human cysteine protease enzymes

	Inhibitor	Enzyme	IC_{50}/nM
1	2-Nap-Leu-N(H)-N_3	Cathepsin K	610
9	2-Nap-Val-Ala-N(H)-N_3	Caspase-1	290
11	Ac-Tyr-Val-Ala-N(H)-N_3	Caspase-1	1700
12	Ac-Asp-Glu-Leu-N(H)-N_3	Caspase-3	7200

These findings of instability to light even in the solid state raise new concerns about applications of alkyl azides in medicine, biology and organic chemistry. Not only should they be stored as solids in the dark, but great care needs to be taken with their handling and use in chemical or biological transformations. The impact of even trace amounts (<1%) of photolytic decomposition products can be profound. For instance, while product **3** only weakly inhibits cysteine proteases such as cathepsin K (IC_{50} > 50 μM), product **2** is such a potent inhibitor (IC_{50} 70 pM) that even <1% contamination can confer substantial enzyme inhibitory potency to samples of >99% pure **1** (Fig. 1)! Similarly, we find that 'aldehyde-free' cysteine protease inhibitor **9** (IC_{50} 290 nM, caspase 1) increases dramatically in potency (IC_{50} 3.8 nM) when exposed to laboratory light for 48 h, correlating with measurement of 4.7% aldehyde contaminant. Pure samples of **1** and **9**, **11** and **12**, synthesized as described[4] but carefully protected from light during synthesis and storage and found to be aldehyde-free by analytical HPLC immediately before and after rapid enzyme assay, also had lower inhibitory potencies against cysteine proteases (Table 1) than reported.[4] While still possessing appreciable inhibitory potency against cysteine protease enzymes (IC_{50} high nM to low μM), their susceptibility to light-mediated decomposition seriously undermines their practical uses in medicine and biology, and can contaminate otherwise valuable uses in chemical transformations.

Notes and references

‡ **Characterization data**. Aldehyde **2**: MS (M + H) = 383; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.60 (s, 1H, CHO), 8.31 (s, 1H, 2-Nap–H), 7.94–7.82 (m, 4H, 2-Nap–H), 7.60–7.54 (m, 2H, 2-Nap–H), 6.71 (m, 2H, Leu-NH, Nle-NH), 4.80 (m, 1H, Leu-α-CH), 4.51 (m, 1H, Nle-α-CH), 1.96–1.72 (m, 4H, Leu-βH, -γH, Nle-βH), 1.64 (m, 1H, Nle-βH), 1.30 (m, 4H, Nle-γH, -δH), 1.03 (d, 3H, J = 6.41 Hz, Leu-δ-CH_3), 1.02 (d, 3H, J = 6.35 Hz, Leu-δ-CH_3), 0.83 (t, 3H, J = 7.01 Hz, Nle-ε-CH_3). Monoacyl aminal **3**: ESI-MS (M + H) = 370; ^{1}H NMR (600 MHz, d_6-DMSO) δ 8.71 (d, 2H, J = 7.94 Hz, Leu-NH and Nle-NH), 8.51 (s, 1H, 2-Nap–H), 8.07 (br. s, 3H, –NH_3), 8.04–7.96 (m, 4H, 2-Nap–H), 7.64–7.59 (m, 2H, 2-Nap–H), 4.88 (m, 1H, Nle-α-CH), 4.60 (m, 1H, Leu-α-CH), 1.82–1.74 (m, 2H, Leu-βH), 1.74–1.68 (m, 1H, Nle-βH), 1.58–1.53 (m, 1H, Leu-γ-CH), 1.39–1.33 (m, 1H, Nle-βH), 1.32–1.26 (m, 4H, Nle-γH, -δH), 0.96 (d, 3H, J = 6.38 Hz, Leu-δ-CH_3), 0.92 (d, 3H, J = 6.43 Hz, Leu-δ-CH_3), 0.87 (t, 3H, J = 6.89 Hz, Nle-ε-CH_3). Amine **8**: MS (M + H) = 384; ^{1}H NMR (600 MHz,

d_6-DMSO) δ 8.70 (d, 1H, J = 7.76 Hz, Leu-NH), 8.50 (s, 1H, 2-Nap–H), 8.01–7.95 (m, 4H, 2-Nap–H), 7.82 (d, 1H, J = 8.63 Hz, Nle-NH), 7.74 (br. s, 2H, –NH_2), 7.64–7.59 (m, 2H, 2-Nap–H), 4.53 (m, 1H, Leu-α-CH), 3.94 (m, 1H, Nle-α-CH), 2.89 (m, 1H, CH–NH_2), 2.80 (m, 1H, CH–NH_2), 1.79 (m, 1H, Leu-βH), 1.72 (m, 1H, Leu-γH), 1.61 (m, 1H, Leu-βH), 1.49 (m, 1H, Nle-βH), 1.39 (m, 1H, Nle-βH), 1.32–1.17 (m, 4H, Nle-γH, -δH), 0.95 (d, 3H, J = 6.55 Hz, Leu-δ-CH_3), 0.92 (d, 3H, J = 6.51 Hz, Leu-δ-CH_3), 0.79 (t, 3H, J = 6.75 Hz, Nle-ε-CH_3).

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