Available online at www.sciencedirect.com

SCIENCE DIRECT®

Tetrahedron

Tetrahedron 61 (2005) 10007–10012

3-Nitro-2-naphthalenemethanol: a photocleavable protecting group for carboxylic acids

Anil K. Singh* and Prashant K. Khade

Department of Chemistry, Indian Institute of Technology, Bombay, Powai, Mumbai 400 076, India

Received 31 May 2005; revised 18 July 2005; accepted 4 August 2005

Abstract—Photocleavable protecting groups are important in synthesis and caging. Among many such groups, 2-nitrobenzyl and related groups have been found useful in many applications. However, most of the known 2-nitrobenzyl-based caging chromophores show either low quantum yield or the photolysis wavelength is not suitable for various applications. In this paper, we report 2-nitro-3-naphthalenemethanol (NNM) as an efficient photocleavable protecting group for molecules containing a carboxylic function. NNM possesses photochemical properties better than the 2-nitrobenzyl chromophores as it is photoactivatable at 380 nm in aqueous medium (CH_3CN/H_2O, 3:2 v/v) showing the desired photochemistry. The carboxylic acids are efficiently photoreleased from NNM-based esters in almost quantitative yield.

1. Introduction

Light-induced deprotection of functional groups is important in synthesis and biomolecular caging.[1–5] For biomolecular caging applications, the photoactivation of the cage is required to be done with high rate and quantum efficiency under physiological conditions at wavelengths that are non-detrimental to biological systems. Several photoactivatable groups such as benzoin, cinnamoyl, coumaryl, 2-nitrobenzyl, phenacyl, polyaromatics etc. have been developed and employed in diverse synthetic and caging applications. Among these, the 2-nitrobenzyl derivatives have attracted much attention because of their compatibility with many functional groups (e.g., phosphate, carboxylates, hydroxyl and amines). Besides its application as an orthogonal protecting group in organic synthesis,[6] it has been extensively used in biology for photoactivation of antibodies,[7,8] synthesis of difficult cyclic peptide,[9] photorelease of Ca^{2+} ions,[10] synthesis of DNA arrays on glass substrates[11] and construction of a light-activated protein by unnatural amino acid mutagenesis.[12] However, most of the known 2-nitrobenzyl-based caging chromophores show either low quantum yield or photolysis wavelength is not suitable for various applications.[3] Recently, we have reported a new caging chromophore namely 2-nitro-3-naphthalenemethanol (NNM, **1**), which possesses photochemical properties better than the 2-nitrobenzyl chromophores.[8] NNM is photoactivatable at 380 nm in aqueous medium (0.9% aqueous NaCl) giving the desired photochemistry with quantum yield of ~0.60. We also demonstrated the application of NNM in protection and deprotection of $-NH_2$ group.[8] The improved photochemical properties of NNM prompted us to examine its usefulness in caging of molecules bearing the carboxyl group.

This paper describes photocleavable protecting group properties of NNM for carboxylic acids. For comparison purpose, we have also synthesized the widely used 4,5-dimethoxy-2-nitrophenyl methanol[3,13] (DMNM, **2**) and examined its efficacy for protection and deprotection of carboxylic acids.

2. Results and discussion

NNM[8] (**1**) and DMNM[14] (**2**) were prepared according to the given procedures. In order to investigate the photocleavable protecting group properties of NNM for carboxylic functional groups, it was attached to four different carboxylic acids to obtain esters **1a–1d** (Scheme 1) and photochemistry of these esters was examined. Similar studies were done on DMNM-based ester **2a** also.

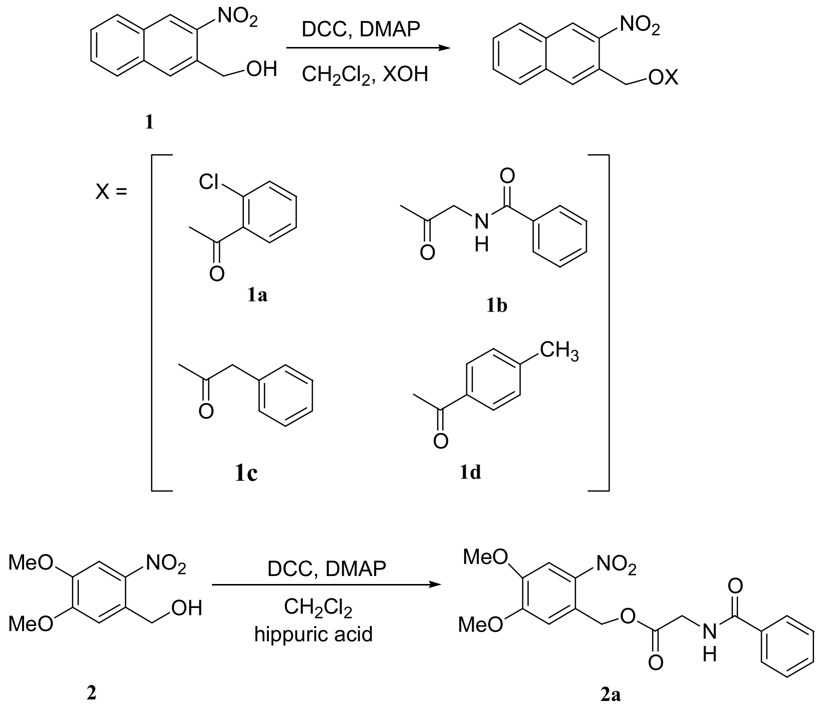
The UV–vis absorption of NNM-based esters (**1a–1d**) is similar to the UV–vis absorption of NNM, with bands located at 214, 258, 302 and 352 nm. Hence, the presence of carboxylic acid moiety in NNM does not change its absorption wavelength. Similarly, the absorption spectrum of ester **2a** is similar to the absorption spectrum of DMNM,

---

Keywords: 3-Nitro-2-naphthalenemethanol; Photocleavable protecting group; Caging.

* Corresponding author. Tel.: +91 22 25767167; fax: +91 22 25767152; e-mail: retinal@chem.iitb.ac.in

doi:10.1016/j.tet.2005.08.019



Scheme 1.

with bands located at 226 and 346 nm. However, a 1.0× 10^{-3} M solution of these esters (**1a**–**1d**, **2a**) in CH_3CN–H_2O (3/2 v/v) shows absorption up to ∼420 nm. Irradiation of a solution of **1a** in CH_3CN–H_2O (3/2 v/v) at ≥370 nm showed no considerable changes at 352 and 304 nm and it decreased at 258 (Fig. 1). When **2a** was photolysed under similar condition, absorbance at 346 nm decreased with concomitant increase at 220 nm (Fig. 2). HPLC analysis of the photomixture of ester **1a** revealed formation of parent carboxylic acids (*o*-chlorobenzoic acid), which can be generated if the ester undergoes the expected known photochemical reaction of NNM (Fig. 3).[8] Similarly, DMNM-based ester **2a** showed photorelease of parent carboxylic acid as evident by HPLC analysis (Fig. 4). This photorelease is again due to the expected photochemical reaction of DMNM.[3] The photochemical mechanism involved in these photoreleases is depicted in Scheme 2. The formation of nitrosoaldehyde is confirmed by proton NMR studies of the photomixture obtained from **1c**. The photomixture shows a peak at 11.20 ppm due –CHO group. It is similar to the mechanism observed in the case of 2-nitrobenzyl-based protecting groups.[2–4,8,15]

HPLC analysis showed formation of a few photoproducts in addition to the expected nirosoaldehyde. These could have generated from the secondary photoreaction of the primary photoproduct nitrosoaldehyde. The nitrosoaldehyde is known to undergo secondary photoreactions like dimerisation and oxidation.[13,16,17] Similar side reactions are also

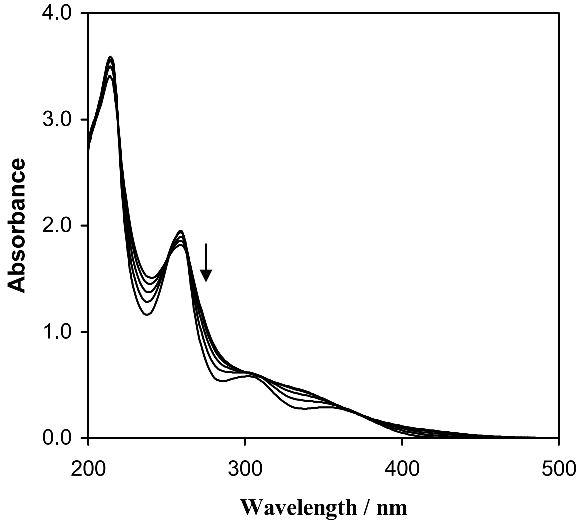


Figure 1. UV–vis absorption spectral changes during photolysis of **1a** (1.0×10^{-4} M) in acetonitrile-water (3/2, v/v).

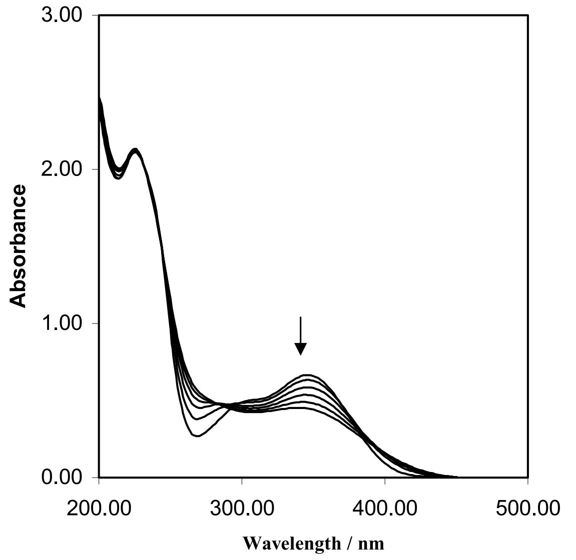


Figure 2. UV–vis absorption spectral changes during photolysis of **2a** (1.0×10^{-4} M) in acetonitrile–water (3/2, v/v).

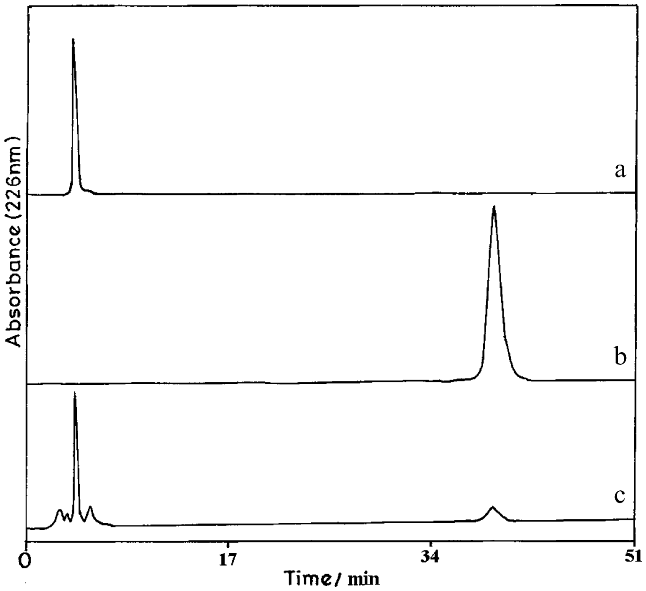


Figure 3. HPLC trace: (a) standard *o*-chlorobenzoic acid, $R_t = 4.3$ min; (b) ester **1a**, $R_t = 37.6$ min; (c) photomixture after 7 h photolysis of ester **1a** (>370 nm) with the released *o*-chlorobenzoic acid.

envisaged in NNM as well. However, these secondary reactions do not affect the percent release of the carboxylic acid, as the release is due to the primary photochemical process.

An action plot was constructed to obtain photodeprotection profiles for the esters. As shown in Figure 5 as a typical example, excellent deprotection yields of 96–100% were obtained in case of esters **1a–1d** (1.0×10^{-3} M) in about 6–7 h of photolysis at >370 nm. However, when **2a** was irradiated under identical conditions the yield of deprotection was 88% but in about 12 h of photolysis. Quantum yields of disappearance (Φ) of the esters **1a–1d** were in range of 0.14–0.16 at 380 nm. However, ester **2a** disappeared under similar photochemical condition with a Φ of only 0.08 (Table 1). The acid moieties on NNM do not significantly affect the photochemical properties of NNM as no considerable differences in the quantum yield of disappearance of esters **1a–1d** have been observed.

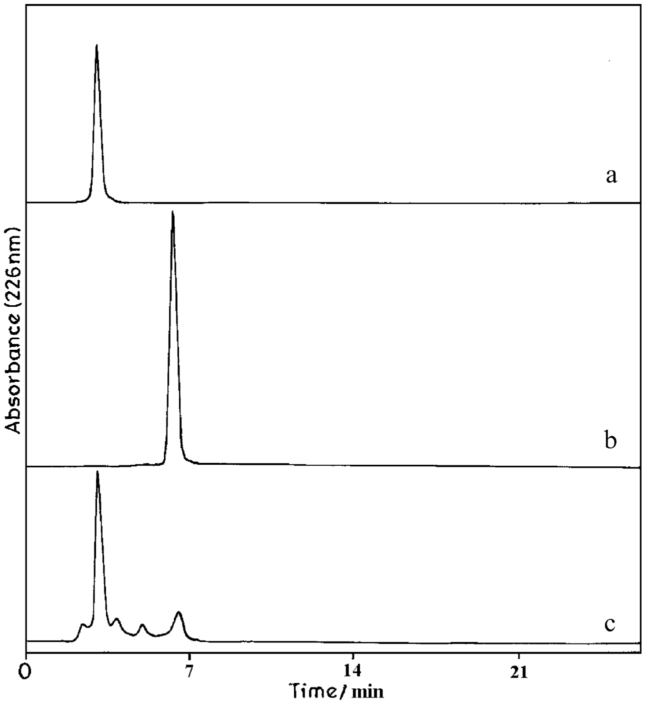
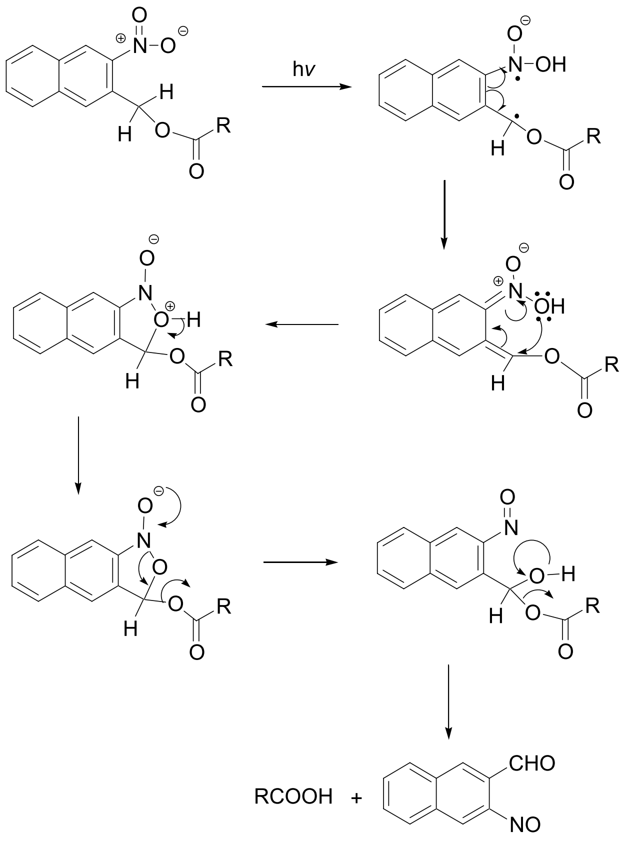


Figure 4. HPLC trace: (a) standard hippuric acid, $R_t = 2.9$ min; (b) ester **2a**, $R_t = 6$ min; (c) photomixture, after 12 h photolysis (>370 nm) of ester **2a** with the released hippuric acid.



Scheme 2.

Photolysis of esters was done in solvents like 1,4-dioxane, ethanol, and acetonitrile. The maximum photorelease

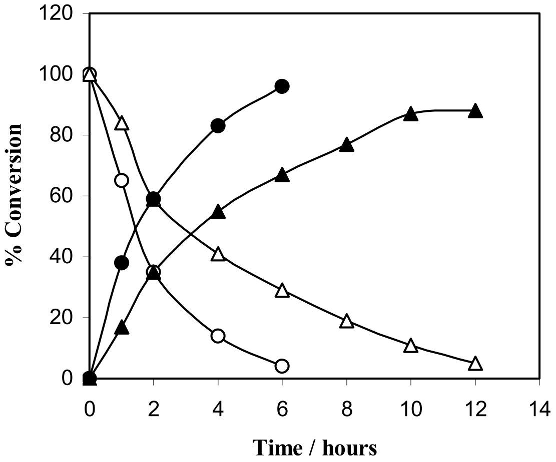
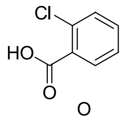
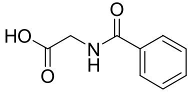
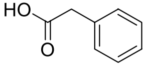
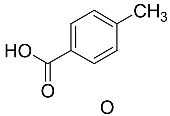
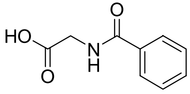


Figure 5. Time dependent decrease of esters (**1b** and **2a**) and release of hippuric acid. Ester **1b** (○); Ester **2a** (△); % Deprotection of hippuric acid from ester **1b** (●); % Deprotection of hippuric acid from ester **2a** (▲).

Table 1. Photochemical properties of esters **1a–1d** and **2a** (1.0×10^{-3} M in 3:2 acetonitrile/water)

Ester	Carboxylic acids	ε at 380 nm ($\mathrm{mol^{-1}\,cm^{-1}\,L}$)	Photolysis time (h)[a]	Unreacted (%)[b]	Deprotection yield (%)[b]	Φ
1a		1626	7	3	97	0.16
1b		1280	6	4	96	0.15
1c		1886	6	0	100	0.15
1d		1755	6	4	96	0.13
2a		3180	12	5	88	0.08

[a] Time of disappearance of ester.
[b] Determined by HPLC.

(~75%) of the acid was observed in acetonitrile. However, the photorelease of acids in 1,4-dioxane and ethanol was only 25 and 35%, respectively. The photorelease of the acids can be further improved by irradiation of the esters in aqueous acetonitrile. Thus, the photoreleases could be maximized to >95% by irradiating the esters in acetonitrile containing water (up to 40%). Thus, polar solvents favour the photorelease. Control experiments showed that no thermal decomposition of the esters occurred as evidenced by HPLC analysis of the samples of esters solutions in $CH_3CN–H_2O$ (3/2 v/v) kept in dark for about 8 days. Thus, NNM can be used to cage molecules bearing carboxyl functions. Its usage for other functional groups like phosphate and alcohol is also envisaged. It may, however, be noted that currently low solubility of NNM in water and additional synthetic work involved in its preparation limit its usage.

3. Conclusions

We have found that NNM is an efficient photocleavable protecting group for the carboxylic acids. It is photoactivatable in aqueous medium at 380–400 nm with the desired photochemical reaction. The carboxylic acids are released by irradiating the corresponding ester solution. Polar solvents like aqueous acetonitrile favour the photorelease of the acid. As compared to DMNM, the photorelease of acids in case of NNM-based esters is almost quantitative and with higher quantum efficiency.

4. Experimental

4.1. General

Starting materials for synthesis were from M/s. Lancaster (UK) and SRL India Ltd. All organic solvents were dried using standard procedures. Petroleum ether (pet-ether, 60–80 °C fraction) was procured in bulk from local suppliers and distilled before use. Other solvents (AR, UV–vis spectroscopic and HPLC grade) were purchased from SRL India Ltd, Mumbai. Thin-layer chromatography (TLC) was performed on silica gel (GF_{254} from SRL India Ltd, Mumbai) plates prepared by coating thin film of silica gel slurry prepared in ethyl acetate on glass plates. Column chromatography was performed on silica gel of 60–120 or 100–200 mesh (SRL India Ltd, Mumbai/Thomas Baker). All the organic extracts were dried over anhydrous sodium sulphate.

Melting points (Mp) were determined with a Veego melting point apparatus and are uncorrected. Electronic absorption spectra were recorded on Shimadzu UV-160 or Shimadzu UV-260 spectrophotometer. Nicolet Impact 400 series Fourier transform spectrometer (FTIR) was used to record the IR spectra of the compounds. NMR spectra were measured in a Fourier transform mode on Varian 300/400 MHz magnetic resonance spectrometer. The high-resolution mass spectra were recorded on Bruker Daltonics APEX 3 T Fourier Transform mass spectrophotometer and Q-Tof micro (YA-105) mass spectrometer. HPLC analyses were done using Hitachi L-6250 intelligent pump attached with U-2000 spectrophotometer under the following conditions: ALTEX ODS 5 μ, 4.6 mm × 25 cm, 55% acetonitrile in water containing 0.1% phosphoric acid, 1.0 mL/min, 226 nm.

Irradiations were done using a 400 W medium pressure mercury lamp (Applied Photophysics Ltd, London UK, Model R-607), fitted either with a f/3.4 monochromator or with a glass filter (≥370 nm having 47 and 96% transmittance at 370 and 420 nm, respectively, with distance between sample and lamp being 2 cm) to isolate the desired wavelength for irradiation. UV–vis spectroscopic grade solvents were used for all photochemical works. The photochemical yield was defined as the ratio of ester molecules disappeared (calculated using HPLC as described above) to the amount of photons absorbed using the potassium ferrioxalate actinometer.[18]

NNM was prepared as described earlier.[8] It is a yellowish solid and remains as such under ambient laboratory light. However, the light yellowish solution of NNM in common organic solvents darkens under ambient laboratory light over a period of time. However, no significant absorption changes are observed in NNM solutions (10^{-3}–10^{-5} M) under ambient laboratory light for about a week.

Esters were prepared according to literature procedure.[19] In a typical case, solution of carboxylic acid (0.24 mmol), alcohol (0.24 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.024 mmol) in anhydrous dichloromethane (5 mL) was stirred for 10 min. To the reaction mixture *N*,*N*-dicyclohexylcarbodiimide (DCC, 0.24 mmol) was added and stirred for 12 h at ambient temperature. Subsequently water (20 mL) was added into reaction mixture and compound was extracted with dichloromethane. The organic layer was washed with sodium bicarbonate solution and then with water and dried with anhydrous sodium sulphate. Solvent evaporation in vacuo gave solid, which was further purified by column chromatography.

4.1.1. 2-Chlorobenzoic acid-3-nitro-naphthalen-2-yl-methyl ester (1a). 2-Chlorobenzoic acid (0.038 g, 0.24 mmol), 3-nitro-naphthalenemethanol (0.049 g, 0.24 mmol) and DMAP (0.003 g, 0.024 mmol) in anhydrous dichloromethane (5 mL) was stirred for 10 min. To the reaction mixture DCC (0.049 g, 0.24 mmol) was added and stirred for 12 h at ambient temperature. Subsequently water (20 mL) was added into reaction mixture and compound was extracted with dichloromethane. The organic layer was washed with sodium bicarbonate solution and then with water and dried with anhydrous sodium sulphate. Solvent evaporation in vacuo gave light brown solid, which was further purified by column chromatography.

Yield: 89%. Mp: 94–96 °C; FTIR (KBr) $\nu_{\max}$ (cm^{-1}): 1729 (OCO), 1525 and 1361 (NO_2); ES-MS: *m/z* 364.0367 ($M^+ + Na$) (found), 364.0353 ($M^+ + Na$) (calcd for $C_{18}H_{12}ClNO_4$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 8.02 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.95–7.89 (m, 2H, Ar-H), 7.73–7.64 (m, 2H, Ar-H), 7.50–7.43 (m, 2H, Ar-H), 7.37–7.33 (m, 1H, Ar-H) and 5.91 (s, 2H, CH_2O).

4.1.2. Benzoylamino acetic acid 3-nitro-naphthalen-2-yl-methyl ester (1b). Yield: 97%; light yellow solid. Mp: 152–154 °C; FTIR (KBr) $\nu_{\max}$ (cm^{-1}): 3293 (NH), 1752 (OCO), 1639 (NHCO), 1530 and 1334 (NO_2); ES-MS: *m/z* 365.1130 ($M^+ + 1$) (found), 365.1137 ($M^+ + 1$) (calcd for $C_{20}H_{16}N_2O_5$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.72 (s, 1H, Ar-H), 8.04–7.93 (m, 3H, Ar-H), 7.85–7.82 (m, 2H, Ar-H), 7.75–7.66 (m, 2H, Ar-H), 7.56–7.43 (m, 3H, Ar-H), 6.72 (br s, 1H, NH), 5.87 (s, 2H, CH_2O) and 4.40 (d, $J = 5.1$ Hz, 2H, CH_2NH).

4.1.3. Phenyl-acetic acid 3-nitro-naphthalen-2-yl-methyl ester (1c). Yield: 63%; light yellow solid. Mp: 90–92 °C; FTIR (KBr) $\nu_{\max}$ (cm^{-1}): 1729 (OCO), 1525 and 1334 (NO_2); ES-MS: *m/z* 344.0911 ($M^+ + Na$) (found), 344.0899 ($M^+ + Na$) (calcd for $C_{19}H_{15}NO_4$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H, Ar-H), 7.97 (d, 1H, Ar-H), 7.72–7.60 (m, 4H, Ar-H), 7.40–7.26 (m, 5H, Ar-H), 5.64 (s, 2H, CH_2O) and 3.78 (s, 2H, CH_2).

4.1.4. 4-Methyl-benzoic acid 3-nitro-naphthalen-2-yl-methyl ester (1d). Yield: 94%; light yellow solid. Mp: 120–122 °C; FTIR (KBr) $\nu_{\max}$ (cm^{-1}): 1716 (OCO), 1539 and 1341 (NO_2); ES-MS: *m/z* 344.0901 ($M^+ + Na$) (found), 344.0899 ($M^+ + Na$) (calcd for $C_{19}H_{15}NO_4$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (s, 1H, Ar-H), 8.06–7.90 (m, 5H, Ar-H), 7.69–7.64 (m, 2H, Ar-H), 7.27 (d, $J = 8.8$ Hz, 2H, Ar-H), 5.87 (s, 2H, CH_2O) and 2.43 (s, 3H, CH_3).

4.1.5. Benzoylamino-acetic acid 4, 5-dimethoxy-2-nitro-benzyl ester (2a). Yield: 63%; light brown solid. Mp: 158–160 °C; FTIR (KBr) $\nu_{\max}$ (cm^{-1}): 3429 (NH), 1741 (OCO), 1659 (NHCO), 1528 and 1327 (NO_2); ES-MS: *m/z* 397.1024 ($M^+ + Na$) (found), 397.1012 ($M^+ + Na$) (cacld for $C_{18}H_{18}N_2O_7$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.54 (t, $J = 7.2$ Hz, 2H, Ar-H), 7.46 (t, $J = 7.2$ Hz, 2H, Ar-H), 7.05 (s, 1H, Ar-H), 6.70 (br s, 2H, NH), 5.65 (s, 2H, CH_2O), 4.36 (d, $J = 4.8$ Hz, 1H, CH_2NH), 4.00 (s, 3H, OCH_3) and 3.96 (s, 3H, OCH_3).

4.2. Photodeprotection of esters and calculation of deprotection yield by HPLC

In a typical experiment, 2 mL of 1.0×10^{-3} M solution of ester in solvents (e.g., acetonitrile–water, 3:2 v/v) was taken in a quartz cuvette. It was photolysed by a 400 W medium pressure mercury lamp and the progress of the photoreaction (deprotection) was monitored by HPLC. For the calculation of percent disappearance of ester and percent appearance of the corresponding acid (e.g., 2-chlorobenzoic acid in case of **1a**), aliquots of 20 μL of the photomixture was removed periodically and analysed by HPLC. The photochemical deprotection yields were calculated by comparing the HPLC trace (peak area) due to released acid (e.g., 2-chlorobenzoic acid in case of **1a**) with the corresponding peak area due to respective standard acid. The HPLC analysis data are average of three to four independent runs.

Acknowledgements

Research grant [01(1509)/98/EMR-II] from the Council of Scientific and Industrial Research, New Delhi, Government of India is gratefully acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.08.019.

References and notes

1. Pillai, V. N. R. *Synthesis* **1980**, 1–26.

2. *Caged compounds*; Marriot, G., Ed.; Methods in Enzymology; Academic: New York, 1998; Vol. 291.
3. Bochet, C. G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125–142.
4. Adams, S. R.; Tsien, R. Y. *Annu. Rev. Physiol.* **1993**, *55*, 755–784.
5. Pellliccioli, A. P.; Wirz, J. *Photochem. Photobiol. Sci.* **2002**, *1*, 441–458.
6. Bochet, C. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 2071–2073.
7. Self, C. H.; Thompson, S. *Nature (Medicine)* **1996**, *2*, 817–820.
8. Singh, A. K.; Khade, P. K. *Bioconjugate Chem.* **2002**, *13*, 1286–1291.
9. Meutermans, W. D. F.; Golding, S. W.; Bourne, G. T.; Miranda, L. P.; Dooley, M. J.; Alewood, P. F.; Smythe, M. L. *J. Am. Chem. Soc.* **1999**, *121*, 9790–9796.
10. Kaplan, J. H.; Ellis-Davies, G. C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6571–6575.
11. McGall, G. H.; Barone, A. D.; Diggelmann, M.; Fodor, S. P. A.; Gentalen, E.; Ngo, N. *J. Am. Chem. Soc.* **1997**, *119*, 5081–5090.
12. Mendel, D.; Ellman, J. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 2758–2760.
13. Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333–6335.
14. Wilcox, M.; Viola, R. W.; Johnson, K. W.; Billington, A. P.; Carpenter, B. K.; McCray, J. A.; Guzikowski, A. P.; Hess, G. P. *J. Org. Chem.* **1990**, *55*, 1585–1589.
15. Corrie, J. E. T.; Barth, A.; Munasinghe, V. R. N.; Trentham, D. R.; Hutter, M. C. *J. Am. Chem. Soc.* **2003**, *125*, 8547–8554.
16. Shamma, M.; Whitesell, J. K.; Warner, P. L., Jr. *Tetrahedron Lett.* **1965**, *6*, 3869–3871.
17. Tanikaga, R. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 210–214.
18. Rabek, J. F. Radiometry and actinometry In *Experimental Methods in Photochemistry and Photophysics*, *Vol. 2*; Wiley: New York, 1982; pp 944–946.
19. Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, *19*, 4475–4478.