Photochemistry

DOI: 10.1002/anie.201402665

Photocaging of Carboxylic Acids: A Modular Approach**

*Wiktor Szymański, Willem A. Velema, and Ben L. Feringa**

Dedicated to the MPI für Kohlenforschung on the occasion of its centenary

Abstract: *Photocaged compounds are important tools for studying and regulating multiple processes, including biological functions. Reported herein is the use of the Passerini multicomponent reaction for modular preparation of photocaged carboxylic acids. The reaction is compatible with several functionalities and proceeds smoothly both in water and dichloromethane. The choice of aldehyde determines the wavelength used for deprotection and enables formation of orthogonally protected products. The isocyanide component can be used for introduction of reactive tags and photosensitizers, as well as for immobilization on a solid support.*

Using molecular approaches for establishing control over biological processes by light is a rapidly advancing research area with numerous potential applications.[1] In particular, introducing photolabile protecting groups into bioactive molecules (photocaging) allows triggering of their activity with light, and is an important tool for controlling biological processes at a molecular level.[1a–c,2] It takes advantage of the unique properties of light, such as low cellular toxicity, the possibility to tune both quantitative (intensity) and qualitative (wavelength) properties, and the high spatiotemporal control over its delivery.

Carboxylic acids are usually caged as photocleavable esters.[3–10] Alternatively, more stable amides of substituted 7-nitroindolines can be used.[11] Photocaging of carboxylic acid moieties has been employed, among other things, for the photochemical control of such biological processes as cell adhesion,[3] activity of the receptors for glycine[4] and GABA$_A$,[5] and glutamate transporters.[6] Photoprotected retinoic acid was reported as a potential tool to study the developmental defects in zebrafish embryos.[7] Further applications include the photoactivation of fluorophores,[8] photocontrolled drug delivery,[9] and release of agrochemicals.[10]

Since water is a solvent that is compatible with bioactive molecules, it would be advantageous to establish a general methodology for the introduction of photolabile protecting groups to carboxylic acid moieties under aqueous conditions. While the formation of esters in water is thermodynamically unfavorable, we envisioned that the use of the Passerini multicomponent reaction[12] could result in the formation of the desired products. In this reaction, which has been performed successfully in water,[13] a carboxylic acid, an aldehyde, and an isocyanide react to form an α-acyloxyamide. The numerous applications[12b–c,14] of the Passerini reaction include the preparation of dendrimer–drug conjugates,[13a] polymers,[14b] and heterocycles.[14c] In combination with enzymatic protocols, it has been used for the preparation of amino acids[14d] and peptides.[14e]

The use of a multicomponent reaction for photocaging bioactive molecules could offer an additional advantage in that it would allow the modular assembly of different functions into the final molecule (Figure 1). In particular, the structure of the aldehyde substrate determines the structure and photochemical properties of the photoactive part of the caged molecule. We envisioned that by appropriately choosing the aldehyde for the reaction, the wavelength of light to be used for deprotection of the carboxylic acid can be tuned. The use of different aldehydes could then allow the preparation of photocaged products,[2] which could be orthogonally deprotected in a mixture. Independently, the isocyanide component introduces a tag, which can be used for visualization (fluorescence), affinity purifications, immobilization on a solid support, or sensitized photodeprotection by intramolecular, photoinduced energy transfer. While there are two examples[5a,15] of using the Passerini reaction for the formation of photolabile esters in dichloromethane, the potential for the modular construction of photoprotected products in water, which stems directly from the multicomponent nature of the Passerini reaction, to the best of our knowledge, has not been explored.

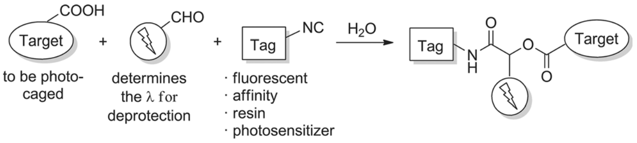


Figure 1. Modular approach for the photocaging of carboxylic acids.

Herein, we present the application of the Passerini reaction, in aqueous and organic media, for the modular preparation of photocaged carboxylic acids, and their application in orthogonal and photosensitized release.

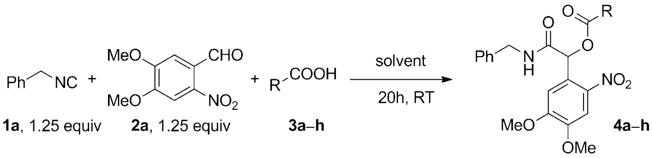
To demonstrate the versatility of this approach with respect to the structure of the carboxylic acid, a model reaction was used (Scheme 1) that employed benzyl isocya-

---

[*] Dr. W. Szymański, W. A. Velema, Prof. Dr. B. L. Feringa
Center for Systems Chemistry, Stratingh Institute for Chemistry
University of Groningen
Nijenborgh 4, 9747 AG, Groningen (The Netherlands)
E-mail: b.l.feringa@rug.nl

[**] We thank Claudia Poloni for insightful discussions. Financial support from and the Ministry of Education, Culture and Science (Gravity program no. 024.001.035), Royal Netherlands Academy of Sciences (KNAW academy chair), and European Research Council (ERC Advanced grant 227897) is gratefully acknowledged.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201402665.



Scheme 1. Passerini reaction of benzyl isocyanide (**1a**), 6-nitroveratraldehyde (**2a**), and carboxylic acids (**3a–h**) to form the products **4**.

nide (**1a**), 6-nitroveratraldehyde (**2a**), and a series of acids (**3a–h**). The reactions were performed in both water and dichloromethane (Table 1). In the reactions of a homologous series of aliphatic carboxylic acids **3a–c** in water (Table 1, entries 1–3), octanoic acid (**3b**) gave the best yield. The lower yield in the case of the more hydrophilic acetic acid (**3a**) has been observed previously in aqueous Passerini reactions.[13d] This effect has been explained[13d] by the fact that the reaction proceeds "on water", and the hydration of acetic acid renders it less effective. We believe that the lower yield in case of the amphiphilic stearic acid (**3c**) could be caused by the fact that it forms micelles and, therefore, is less accessible to the other reactants. Results obtained in CH_2Cl_2 are complementary to those obtained in water, as products **4a** and **4c** could be obtained in higher yields.

Table 1: Yields of the isolated products **4** from the Passerini reaction with a series of carboxylic acids in water and dichloromethane.

Entry	R	Product	Water [%][a]	CH_2Cl_2 [%][a]
1	CH_3	**4a**	25	86
2	$CH_3(CH_2)_6$	**4b**	85	50
3	$CH_3(CH_2)_{16}$	**4c**	<10	48
4	$PhCH_2$	**4d**	89	77
5	$FmocNHCH_2$	**4e**	56	<10
6	Ph	**4f**	73	72
7	$4\text{-}MeOC_6H_4$	**4g**	40	52
8	$4\text{-}FC_6H_4$	**4h**	85	84

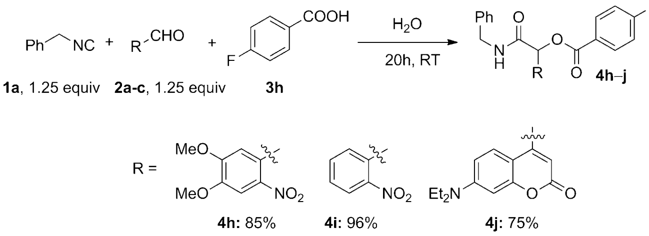
[a] reaction conditions: **1a** (0.125 M), **2a** (0.125 M), and **3** (0.100 M), solvent (water or CH_2Cl_2), 20 h, RT. Fmoc = 9-fluorenylmethoxycarbonyl.

The photocaged phenylacetic acid **4d** was obtained in high yield in both reaction media (Table 1, entry 4). When the Fmoc-protected glycine **3e** was used as a substrate (Table 1, entry 5), the product was formed only in the reaction run in water. For the conversions of the substituted benzoic acids **3f–h**, similar trends were observed in water and CH_2Cl_2, that is, the highest yield was obtained for the fluoro-substituted substrate **4h**, and the lowest for methoxy-substituted compound **4g**. Hydrophobic effects have been suggested to contribute to the rate acceleration of Passerini reactions in water[13b] and higher yields were observed when more hydrophobic reactants were used.[13d] This trend correlates well with our observation that good yields can be obtained for the conversions of carboxylic acids containing hydrophobic residues (Table 1, entries 2, 4–6, and 8). This trend is also reflected in the changes in the outcome of the reactions using substituted benzoic acids (Table 1, entries 6–8), wherein the substitution with hydrophobic fluorine gives the best yield (Table 1, entry 8) and substitution with a methoxy group (Table 1, entry 7) results in a lower yield as compared to the unsubstituted benzoic acid (Table 1, entry 6).

The uncaging of *o*-nitrobenzyl derivatives results in formation of reactive species,[16] which might react with reaction products and lower their yield. Therefore we followed the deprotection of **4d** (ca. 15 mM), under $\lambda = 365$ nm irradiation, by ^1H NMR spectroscopy with TMS as an internal standard (see the Supporting Information). After 18 hours of irradiation, we observed a greater than 95% conversion of the substrate, and the product was obtained in 91% analytical yield, which indicates that the uncaging is a selective process.

The prospects offered by wavelength-selective, orthogonal uncaging of two or more photoprotected, bioactive compounds have recently attracted considerable attention.[17] Since the initial report in 2000,[17a] this concept has been used for the independent release of glutamate/GABA on living neurons[17b] and for chromatically selective uncaging of nucleotides,[17c] and cAMP,[17d] among others. The major advantages of using multiple orthogonally photoprotected compounds stem from the possibility to selectively trigger various responses from the studied biological system. This ability is particularly useful in cases where two independent mechanisms are involved in the on/off triggering of a biological process.[17d]

Since in the presented approach the wavelength used for the deprotection is defined by the structure of the aldehyde (Figure 1), we studied the possibility of applying different aldehydes to obtain orthogonally photoprotected products (Scheme 2). Besides 6-nitroveratraldehyde (**2a**), 2-nitrobenzaldehyde (**2b**) and the coumarin-derived aldehyde **2c** were employed as substrates. We were delighted to observe that for all the aldehydes **2a–c** the products **4h–j** were isolated in good yields (Scheme 2). As expected, the obtained products differed considerably in their spectral properties, with compound **4i** showing the most blue-shifted spectrum ($\lambda_{max} <$ 300 nm) and compound **4j** showing an absorption that extended into the visible range. UV-vis spectroscopy measurements confirmed the uncaging of the compounds **4h–j** by irradiation with light of different wavelengths, both in chloroform and in aqueous phosphate buffer (See the Supporting Information).



Scheme 2. Preparation of the protected acids **4h–j** for chromatically selective uncaging.

By taking advantage of the differences in photochemical properties of the orthogonally photoprotected compounds

4h–j, we attempted a selective uncaging of **4j** in the presence of **4i**. Irradiation with white light (Figure 2, first 3 h) resulted in a clean and almost complete conversion of **4j** into the uncaged 4-fluorobenzoic acid (**3h**), while the concentration of **4i** remained stable. Subsequent irradiation with UV light allowed the deprotection of **4i**. These results highlight the potential of applying the aqueous Passerini reactions to the preparation of orthogonally protected carboxylic acids.

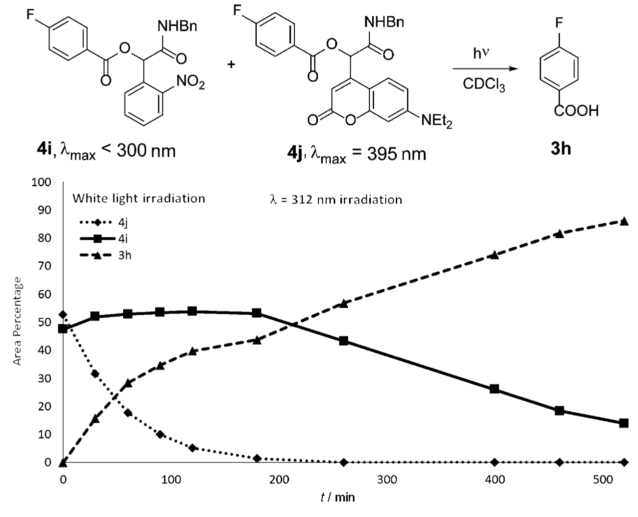
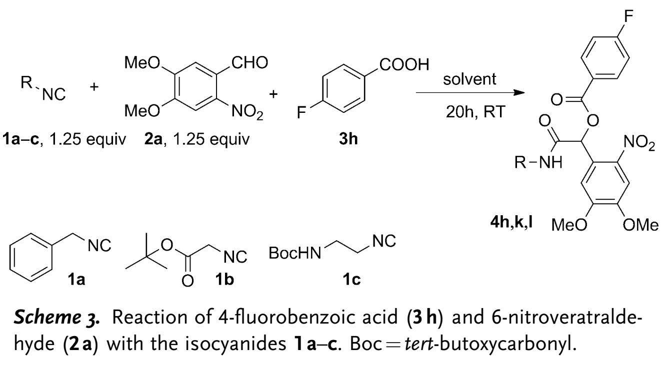


Figure 2. Selective deprotection of orthogonally protected **4i** (≈7 mm) and **4j** (≈7 mm) in $CDCl_3$. Studied by ^{19}F NMR spectroscopy. See the Supporting Information for NMR spectra.

The isocyanide component is used in our system as a highly reactive reagent which enables the ester formation in water. However, further opportunities emerge if this component carries a tag which could be used purposes such as purification, immobilization on solid support, photosensitizing, or further functionalization.

With this in mind, we studied the model reaction of various isocyanides with 4-fluorobenzoic acid (**3h**) and 6-nitroveratraldehyde (**2a**; Scheme 3 and Table 2). The best results were obtained when benzyl isocyanide was used as a starting material (Table 2, entry 1), and this we consider to be the reactant of choice for reactions both in water and in CH_2Cl_2, if no tag is required in the final photoprotected molecule. We also explored the reactions with the isocyanides **1b** and **1c**. In both cases, the Passerini reaction gave a moderate yield in water and a better result in CH_2Cl_2 (Table 2, entries 2 and 3). Notably, the products **4k** and **4l** contain groups with the potential for further functionalization, namely a protected carboxylic acid and amine moieties (respectively).



Scheme 3. Reaction of 4-fluorobenzoic acid (**3h**) and 6-nitroveratraldehyde (**2a**) with the isocyanides **1a–c**. Boc = *tert*-butoxycarbonyl.

Table 2: Yields of isolated products from the Passerini reaction with a series of isocyanides.

Entry	Substrate	Product	Water [%][a]	CH_2Cl_2 [%][a]
1	**1a**	**4h**	85	84
2	**1b**	**4k**	35	56
3	**1c**	**4l**	38	86

[a] reaction conditions: **1** (0.125 m), **2a** (0.125 m), and **3h** (0.100 m), solvent (water or CH_2Cl_2), 20 h, RT.

We further explored the possibility of using the isocyanide component for photocleavable immobilization of carboxylic acids on a solid support (Figure 3). The photorelease of resin-bound carboxylic acids has been used in solid-phase peptide synthesis.[2,18] We studied the Passerini reaction involving the isocyanide-decorated polystyrene resin **C** (Figure 3), which was prepared from an aminomethylated polystyrene resin (**A**) using a published procedure.[19] Coupling of the amine groups of **A** to formic acid with subsequent formamide dehydration yielded the resin **C**, as indicated by the formation of IR stretching bands at 1694 cm^{-1} (**B**, formamide C=O) and 2146 cm^{-1} (**C**, isocyanide; For IR spectra, see the Supporting Information).[19b]

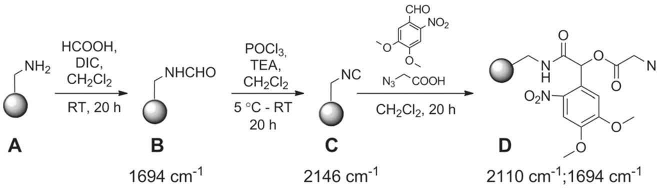
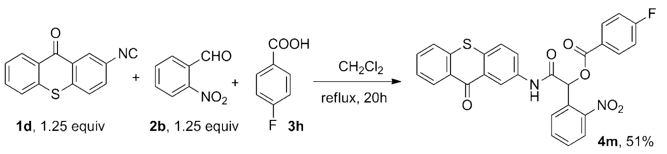


Figure 3. Preparation of the isocyanide-modified resin **C** and its application in a model Passerini reaction to immobilize azidoacetic acid on a resin with a photocleavable linker (**D**). For experimental details and IR spectra, see the Supporting Information. DIC = diisopropylcarbodiimide, TEA = triethylamine.

A Passerini reaction with the resin **C** was performed in CH_2Cl_2, since it is a solvent compatible with the type of resin used in this experiment. Azidoacetic acid was used as the acid component, since the azide can be conveniently used as an IR marker as a result of its characteristic and strong absorbance. Finally, 6-nitroveratraldehyde (**2a**) was used to install a photoresponsive moiety. Conversion into the resin **D** (Figure 3) was confirmed by the disappearance of the isocyanide stretch and emergence of the azide band at 2110 cm^{-1} in the IR spectrum (in neat azidoacetic acid: 2108 cm^{-1}), and the carbonyl group stretches at about 1690 cm^{-1}. This protocol constitutes a general method for the immobilization of carboxylic acids on resins with photolabile linkers, whose spectral properties could be controlled by the choice of the aldehyde component.

The introduction of auxiliary chromophores to the photoprotected compound is an attractive strategy which helps to improve the efficiency of the deprotection by photoinduced energy transfer and allows the use of light with a longer wavelength.[2] The 9H-thioxanthen-9-one moiety has been used successfully as a photosensitizer[20] and a detailed mechanism for the energy transfer to the *o*-nitrobenzyl group was proposed.[20b] However, the covalent introduction of a photosensitizer, while offering a way to tune the photochemical properties of the photoactive group, is limited by the tedious synthesis.[20b] Therefore we envisioned that a multicomponent procedure, which brings together the target compound and both the primary and auxiliary chromophores in one step, would offer an unparalleled access to such compounds.

We tested the possibility of using the isocyanide **1d** (Scheme 4), derived from 9*H*-thioxanthen-9-one, in the Passerini reaction. The isocyanide **1d** can be prepared in one step from the respective aniline (see the Supporting Information). However, it was found that the use of **1d** is limited by its low solubility, and the formation of the product **4m** was observed only when CH_2Cl_2 was used as the solvent, after an overnight reaction at an elevated temperature.



Scheme 4. Reaction of 4-fluorobenzoic acid (**3h**) and 2-nitrobenzaldehyde (**2a**) with the 9*H*-thioxanthen-9-one-based isocyanide **1d**.

A comparison of the UV-vis spectra (See the Supporting Information) of **4m** and its analogue **4i**, a 2-nitrobenzyl ester which has no photosensitizer in its structure, revealed that **4m** has a much higher absorption within the range of wavelengths studied. In particular, it shows a strong absorption at wavelengths greater than $\lambda = 400$ nm, whereas the absorption of **4i** is negligible. With this in mind, we studied the release of **3h** in a roughly equimolar mixture of **4m** and **4i** (Figure 4, upper panel) upon irradiation with light of different wavelengths. Irradiation at $\lambda = 312$ nm (Figure 4, middle panel) resulted in clean release of **3h** from **4m**, about three times faster when compared to release from **4i**. The use of white light (Figure 4, lower panel), in contrast, clearly showed the influence of the secondary chromophores as it allowed the selective cleavage of **4m**, while the concentration of **4i** remained unchanged. Despite the moderate yield of **4m** in the Passerini reaction (Scheme 4), we find this an attractive, one-step alternative to the multistep approaches used in the preparation of caged compounds with the possibility of photosensitized release. As long as respective isocyanides can be conveniently prepared, this methodology may be useful for the introduction of various photosensitizers with different photochemical properties.

In summary, we present here the application of the Passerini reaction for the introduction of photocleavable groups to carboxylate moieties. The reaction can be performed both in organic (dichloromethane) and aqueous environment. The multicomponent character of the presented procedure allows the modular assembly of the products in one step. In particular, the aldehyde component dictates the photochemical nature of the final product and determines the wavelength of light that can be used for deprotection. Finally, the isocyanide component can be used to introduce a diversity of tags to the protected product. We have explored, in detail, the possibility of using it for the photoreversible immobilization on the solid support and for the covalent introduction of a photosensitizer, which allows tuning of the photochemical properties of the final compounds and deprotection with visible light.

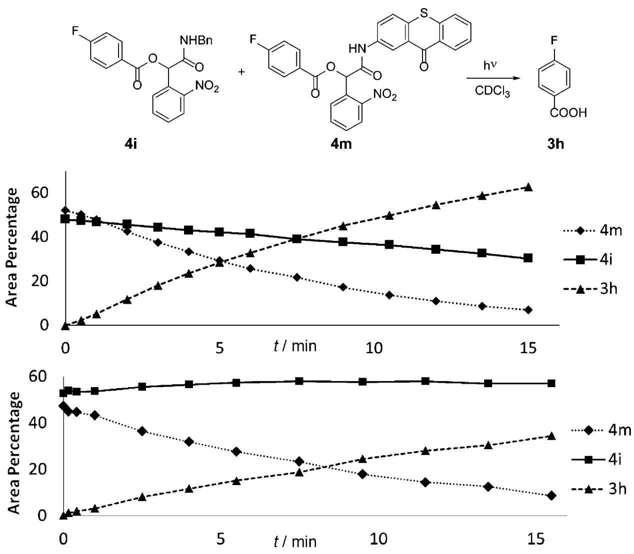


Figure 4. Photosensitized release of **3h** from a mixture of **4i** (≈8 mM) and **4m** (≈8 mM) in $CDCl_3$. Studied by ^{19}F NMR spectroscopy. Upper graph: irradiation at $\lambda = 312$ nm. Lower graph: irradiation with white light. See the Supporting Information for NMR spectra.

Received: February 21, 2014
Revised: April 5, 2014

Keywords: multicomponent reactions · photocaging · protecting groups · synthetic methods · water chemistry

---

[1] a) G. Mayer, A. Heckel, *Angew. Chem.* **2006**, *118*, 5020–5042; *Angew. Chem. Int. Ed.* **2006**, *45*, 4900–4921; b) D. D. Young, A. Deiters, *Org. Biomol. Chem.* **2007**, *5*, 999–1005; c) A. Deiters, *ChemBioChem* **2010**, *11*, 47–53; d) A. A. Beharry, G. A. Woolley, *Chem. Soc. Rev.* **2011**, *40*, 4422–4437; e) C. Brieke, F. Rohrbach, A. Gottschalk, G. Mayer, A. Heckel, *Angew. Chem.* **2012**, *124*, 8572–8604; *Angew. Chem. Int. Ed.* **2012**, *51*, 8446–8476; f) W. Szymanski, J. M. Beierle, H. A. V. Kistemaker, W. A. Velema, B. L. Feringa, *Chem. Rev.* **2013**, *113*, 6114–6178; g) Q. Liu, A. Deiters, *Acc. Chem. Res.* **2014**, *47*, 45–55.

[2] a) H.-M. Lee, D. R. Larson, D. S. Lawrence, *ACS Chem. Biol.* **2009**, *4*, 409–427; b) Q. Shao, B. Xing, *Chem. Soc. Rev.* **2010**, *39*, 2835–2846; c) P. Klán, T. Šolomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov, J. Wirz, *Chem. Rev.* **2013**, *113*, 119–191.

[3] C. A. Goubko, A. Basak, S. Majumdar, H. Jarrell, N. H. Khieu, X. Cao, *J. Biomed. Mater. Res. A* **2013**, *101A*, 787 – 796.

[4] C. Grewer, J. Jäger, B. K. Carpenter, G. P. Hess, *Biochemistry* **2000**, *39*, 2063 – 2070.

[5] a) L. Fan, R. W. Lewis, G. P. Hess, B. Ganem, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3932 – 3933; b) R. W. Lewis, G. P. Hess, B. Ganem, *Future Med. Chem.* **2011**, *3*, 243 – 250.

[6] K. Takaoka, Y. Tatsu, N. Yumoto, T. Nakajima, K. Shimamoto, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 965 – 970.

[7] P. Neveu, I. Aujard, C. Benbrahim, T. Le Saux, J.-F. Allemand, S. Vriz, D. Bensimon, L. Jullien, *Angew. Chem. Int. Ed.* **2008**, *47*, 3744 – 3746; *Angew. Chem.* **2008**, *120*, 3804 – 3806.

[8] R. H. Pawle, V. Eastman, S. W. Thomas, *J. Mater. Chem.* **2011**, *21*, 14041 – 14047.

[9] M. W. Grinstaff, P. Barthelemy, C. Prata, L. Moreau, US2006/241071A1, **2006**.

[10] D. A. Morgenstern, WO2013/55944A2, **2013**.

[11] a) B. Amit, D. A. Ben-Efraim, A. Patchornik, *J. Am. Chem. Soc.* **1976**, *98*, 843 – 844; b) G. Papageorgiou, D. C. Ogden, A. Barth, J. E. T. Corrie, *J. Am. Chem. Soc.* **1999**, *121*, 6503 – 6504; c) J.-L. Débieux, C. G. Bochet, *J. Phys. Org. Chem.* **2010**, *23*, 272 – 282.

[12] a) M. Passerini, L. Simone, *Gazz. Chim. Ital.* **1921**, *51*, 126 – 129; b) A. Dömling, I. Ugi, *Angew. Chem. Int. Ed.* **2000**, *39*, 3168 – 3210; *Angew. Chem.* **2000**, *112*, 3300 – 3344; c) A. Dömling, *Chem. Rev.* **2006**, *106*, 17 – 89.

[13] a) A. E. J. de Nooy, G. Masci, V. Crescenzi, *Macromolecules* **1999**, *32*, 1318 – 1320; b) M. C. Pirrung, K. Das Sarma, *J. Am. Chem. Soc.* **2004**, *126*, 444 – 445; c) M. A. Mironov, M. N. Istvanova, M. I. Tokareva, V. S. Mokrushin, *Tetrahedron Lett.* **2005**, *46*, 3957 – 3960; d) T. Sela, A. Vigalok, *Adv. Synth. Catal.* **2012**, *354*, 2407 – 2411.

[14] a) Z. Y. Du, Y. H. Lu, X. D. Dai, D. Zhang-Negrerie, Q. Z. Gao, *J. Chem. Res.* **2013**, *37*, 181 – 185; b) O. Kreye, T. Tóth, M. A. R. Mayer, *J. Am. Chem. Soc.* **2011**, *133*, 1790 – 1792; c) Q. Xia, B. Ganem, *Org. Lett.* **2002**, *4*, 1631 – 1634; d) W. Szymanski, R. Ostaszewski, *Tetrahedron: Asymmetry* **2006**, *17*, 2667 – 2671; e) W. Szymanski, M. Zwolinska, R. Ostaszewski, *Tetrahedron* **2007**, *63*, 7647 – 7653.

[15] L. Li, A. Lv, X.-X. Deng, F.-S. Du, Z.-C. Li, *Chem. Commun.* **2013**, *49*, 8549 – 8551.

[16] A. P. Pelliccioli, J. Wirz, *Photochem. Photobiol. Sci.* **2002**, *1*, 441 – 458.

[17] a) C. G. Bochet, *Tetrahedron Lett.* **2000**, *41*, 6341 – 6346; b) S. Kantevari, M. Matsuzaki, Y. Kanemoto, H. Kasai, G. C. R. Ellis-Davies, *Nat. Methods* **2010**, *7*, 123 – 125; c) A. Rodrigues-Correia, X. M. M. Weyel, A. Heckel, *Org. Lett.* **2013**, *15*, 5500 – 5503; d) J. P. Olson, M. R. Banghart, B. L. Sabatini, G. C. R. Ellis-Davies, *J. Am. Chem. Soc.* **2013**, *135*, 15948 – 15954; e) V. San Miguel, C. G. Bochet, A. del Campo, *J. Am. Chem. Soc.* **2011**, *133*, 5380 – 5388; f) J.-L. Débieux, C. G. Bochet, *Chem. Sci.* **2012**, *3*, 405 – 406.

[18] a) F. Guillier, D. Orain, M. Bradley, *Chem. Rev.* **2000**, *100*, 2091 – 2158; b) A. Ajayaghosh, V. N. R. Pillai, *J. Org. Chem.* **1987**, *52*, 5714 – 5717; c) C. Marinzi, J. Offer, R. Longhi, P. E. Dawson, *Bioorg. Med. Chem.* **2004**, *12*, 2749 – 2757.

[19] a) J. J. Chen, A. Golebiowski, J. McClenaghan, S. R. Klopfenstein, L. West, *Tetrahedron Lett.* **2001**, *42*, 2269 – 2271; b) N. R. Aguiam, V. I. Castro, A. I. F. Ribeiro, R. D. V. Fernandes, C. M. Carvalho, S. P. G. Costa, S. M. M. A. Pereira-Lima, *Tetrahedron* **2013**, *69*, 9161 – 9165.

[20] a) D. Wöll, S. Walbert, K. P. Stengele, T. J. Albert, T. Richmond, J. Norton, M. Singer, R. D. Green, W. Pfleiderer, U. E. Steiner, *Helv. Chim. Acta* **2004**, *87*, 28 – 45; b) D. Wöll, S. Laimgruber, M. Galetskaya, J. Smirnova, W. Pfleiderer, B. Heinz, P. Gilch, U. E. Steiner, *J. Am. Chem. Soc.* **2007**, *129*, 12148 – 12158.