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The all-photochemical synthesis of an OGP(10–14) precursor†

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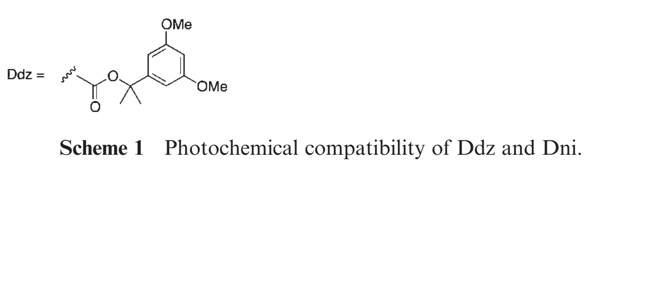
An all-photochemical synthesis of OGP(10–14), the biologically active part of an osteogenic growth peptide, was performed. This required the preparation of photochemically activable amino acids containing a 5,7-dinitroindoline derivative on the C-terminus and a photolabile Ddz protecting group on the N-terminus. The photochemical acyl transfer from the dinitroindoline to an incoming nucleophilic amino group, which creates the amide bond, requires an irradiation wavelength of 385 nm. The deprotection of the Ddz group requires a shorter wavelength (300 nm), thus making both successive processes compatible.

Peptide synthesis goes back to the early ages of organic synthesis, but it was only with the advent of solid-phase synthesis that it became a major and now routine strategy.[1] Countless polymeric supports, linkers, protecting groups, coupling agents, automation and reaction conditions have been optimized over the years, and the degree of performance is now reaching near-perfection. However, all the methods currently available (with the notable exception of enzymatic or chemical ligation)[2] rely on the use of reactive species in solution, either as an electrophilic acyl donor, or a coupling agent which will convert *in situ* a carboxylic acid into an acyl donor. Furthermore, the classical methods invariably require protection of the activated building block amine function, which needs to be removed after the coupling step using relatively harsh reagents (trifluoroacetic acid or piperidine for the most widely used Boc and Fmoc protecting groups). Hence these methods are not suitable for applications where the long-term storage of highly reactive species is precluded, such as *in vivo* implanted microfluidic devices, remotely controlled experiments in extreme locations (space or undersea) *etc.* In such situations, the use of thermally unreactive reaction partners that could be activated on-demand by an external trigger (*e.g.* by switching on a light source) constitutes an attractive alternative. In this edge article, we will provide the proof of principle of this concept by an all-photochemical synthesis of a precursor of the biologically active part of OGP, an osteogenic growth pentapeptide, without the need of an external reagent for the coupling or the removal of protecting groups.

Based on initial work by Pass *et al.*,[3] we and others have shown that *N*-acylated nitroindoline derivatives undergo a smooth photoinduced acyl transfer in the presence of a nucleophile,[4] such as an amine[5] or an alcohol.[6] This process requires wavelengths typically longer than 375 nm. On the other hand, photochemical removal of the acid-labile Ddz protecting group occurs at wavelengths shorter that 350 nm.[7] This is a typical case of chromatically modulated lability.[8] This was checked experimentally, by the reaction of Ddz-protected-L-leucine derivative **1** with phenethylamine **2**, under irradiation at 385 nm with a LED-based reactor for 16 h in acetonitrile, whereby α-amino amide **3** was obtained in 92% yield (Scheme 1).

Having established the compatibility of the photochemical amide formation in the presence of a Ddz protecting group, and having dinitroindoline derivatives of most proteinogenic amino acids in hand,[9] we decided to check whether a dipeptide could be assembled by successive photochemical acylation at long wavelength, followed by deprotection at shorter wavelength. Thus, Ddz-L-Leu-Dni (Dni = 5,7-dinitroindolinyl) derivative **1** was irradiated at 375 nm in acetonitrile in the presence of phenylalanine ester hydrochloride **4** for 17 h in an LED-based reactor (Scheme 2). The *N*-protected dipeptide ester **5** was obtained in a good yield (81%), and no racemization was detected by ^{1}H-NMR. In order to make sure that racemization would be detectable, a control experiment by the same reaction between L- and D-alanine derivatives **7** and *ent*-**7** with **4** was performed in the LED reactor; the diastereoisomers **8** and **9** were obtained, showing markedly different NMR signals for the methyl groups.



Scheme 1 Photochemical compatibility of Ddz and Dni.

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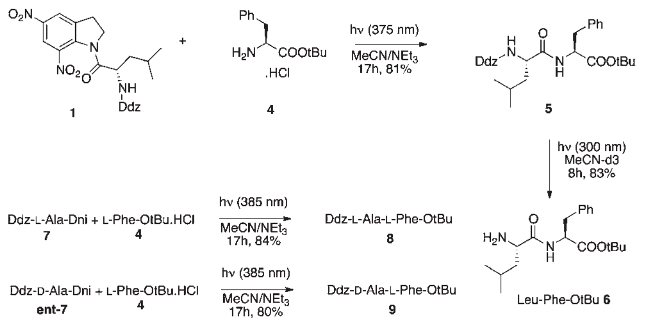
† Electronic supplementary information (ESI) available: Complete experimental details and copies of spectra for all new compounds.

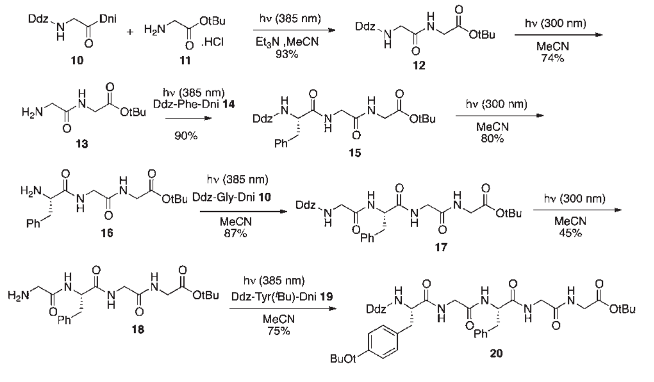


Scheme 2 Photochemical formation of dipeptides.

Subsequent irradiation of **5** at 300 nm using a Rayonet® reactor for 8 h gave the dipeptide ester **6** in a 83% yield.

Encouraged by the success of this elementary step, we decided to prepare a "real" peptide by this technique. The osteogenic growth peptide (OGP) is a naturally occurring tetradecapeptide. It was shown that a synthetic peptide containing the Tyr-Gly-Phe-Gly-Gly subsequence at the C-terminus retained a similar biological activity.[10] Thus, we started by binding glycine *tert*-butyl ester hydrochloride (**11**) to another Ddz-protected glycine **10** by our photoacylation reaction, in the presence of triethylamine to liberate *in situ* the free base. A high yield was obtained after isolation. Removal of the Ddz group was effected by irradiation at 300 nm in a Rayonet® reactor, and the next amino acid, a Ddz-protected phenylalanine, was introduced photochemically, followed by deprotection. The sequence was repeated once more with another glycine derivative, **10**, and finally *O-tert*-butyl-*N*-Ddz protected tyrosine was attached photochemically in a 75% yield to give the pentapeptide precursor of OGP(10–14) (Scheme 3). Here again, spectroscopic analysis by ^{1}H and ^{13}C NMR showed only a single diastereoisomer and a single peptide sequence.

All the intermediates were isolated, purified by chromatography and characterized (see ESI†). We also tested an *in situ* protocol, where no purifications were performed. Thus, Ddz-L-Leu-Dni **1** was irradiated for 12 h in the presence of L-leucine *tert*-butyl ester hydrochloride and triethylamine at 385 nm, followed by irradiation at 300 nm for 8 h. Addition of another portion of **1**, followed by both irradiations gave a tripeptide. Final addition of another portion of **1** gave a crude mixture, which contained the Ddz-tetraleucine *tert*-butyl ester, together with tri- and dileucine derivatives. Clearly, additional optimization would be required for a no-intervention protocol to succeed.



Scheme 3 The all photochemical synthesis of the OGP(10–14) precursor.

In conclusion, we were able to prepare a pentapeptide from each elementary building block without the need for additional reagents. Although this strategy by no means competes with classical peptide synthesis in terms of yields and costs, it highlights the power of wavelength selective photochemical reactions, and sets the stage for specific applications where scale and cost are not critical, but where no aggressive reagents are tolerated. Such an application could be *in situ* peptide synthesis of pharmacological relevance on microchips, or even within biological media.

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