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Wavelength dependent photo-controlled differential release of compounds from solid phase resin

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A method to effect photo-mediated differential release of bead-based compound libraries using a tuneable laser in combination with chromatically orthogonal photolabile linkers is described.

The high throughput screening (HTS) of synthetic combinatorial compound libraries is now well established within the pharmaceutical industry as a means of identifying new starting points for medicinal chemistry programmes.[1] Such libraries may be rapidly generated as mixed 'pools' of compounds by utilising 'split and mix' solid phase synthesis techniques.[2] Although various screening strategies may then be adopted, an approach based upon tiered compound release is particularly efficient. Following partial cleavage from resin, the pooled compounds are first screened to identify active pools.[3] Individual 'hits' are then identified by separating the partially cleaved beads from the active pools into discrete wells, and then cleaving and screening the remaining resin-bound material.

In practice, differential compound release may be most reliably achieved by invoking the sequential *exhaustive* cleavage of material immobilised on bead through two orthogonal linkers.[4] To avoid the need to add noxious exogenous reagents or use harsh conditions (*e.g.* TFA) to effect linker cleavage, the use of two orthogonal photolabile linkers represents a particularly desirable combination.[5] Recently, the feasibility of wavelength dependent orthogonal photocleavage has been reported by Bochet and co-workers.[6]

Herein, we describe our own independent work in this area and demonstrate for the first time the photo-controlled differential release of a compound array of carboxylic acids from solid phase resin. This was achieved using beads bifurcated with a combination of nitroveratryl (NV) **1**[7] and pivaloyl glycol (PG) **2**[8] photo-linkers, whereby the desired orthogonality is obtained primarily by changing the wavelength of the incident monochromatic light (Fig. 1). In contrast to the NV linker **1**, the ketonic PG linker **2** does not absorb at higher wavelengths and we anticipated it would therefore be photo-stable above approximately 330 nm, giving rise to the desired photo-orthogonality.

Experimentally, amino-Tentagel™ resin was derivatised with the NV linker **1** and the PG linker **2**, and these resins were acylated with naphthalene-3-propanoic acid to afford the resins **3** and **4**. Each resin was then distributed as pools of 20 beads in a 96 well microtitre beadcup plate.[4a] The beads were suspended in DMSO (20 μl per well) and the individual pools were sequentially subjected to pulsed laser photolysis at constant incident energy (E = 75 μJ pulse^{-1}, t = 5 min) varying only the wavelength of the incident radiation. A narrow bandwidth tuneable research laser was used to carefully control both the wavelength and energy of the incident irradiation.[9] The supernatant solutions obtained were analysed by LC-MS.

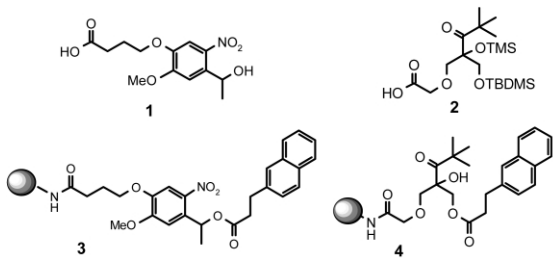

Fig. 1 Nitroveratryl (NV) and pivaloyl glycol (PG) linkers and resins for differential photo-release studies.

As shown in Fig. 2, under the irradiation conditions employed, the NV linker **1** undergoes photocleavage over a broad bandwidth with maximum cleavage rates at 320 nm and 340 nm. In contrast, the PG linker **2** is photo-stable above 340 nm. Additionally, under the experimental conditions, the maximum rate of cleavage of the PG resin **4** is significantly higher than the corresponding NV resin **3**.

These results confirmed that orthogonal photo-mediated differential release could be reliably achieved by using a resin bifurcated with NV and PG linkers, promoting exhaustive photocleavage first at 355 nm, and then at 300 nm. In both instances, the ratio of material released from the resin would be determined solely by the ratio of the two linkers present on bead.

To establish suitable general conditions for exhaustive cleavage, we prepared the bifurcated resins **5** and **6** differentially derivatised with the PG and NV linkers in a 1 : 2 ratio[10] (Fig. 3). These were subjected to laser photolysis under a range of conditions setting the wavelength at either 355 or 300 nm respectively. In this way, exhaustive cleavage was found to be

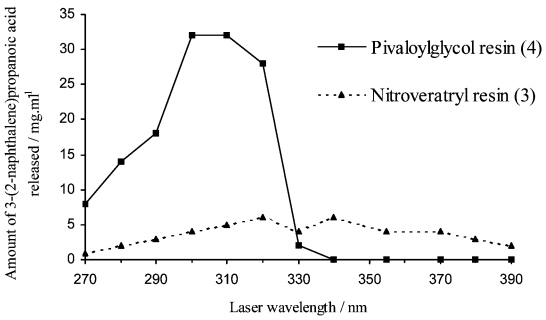

Fig. 2 Comparison of photolysis characteristics of NV and PG photolinkers under irradiation at 10 Hz, 75 μJ pulse^{-1}, t = 5 min.

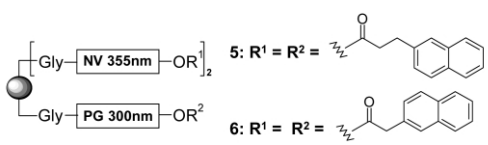

Fig. 3 Bifurcated resins used to optimise photocleavage conditions.

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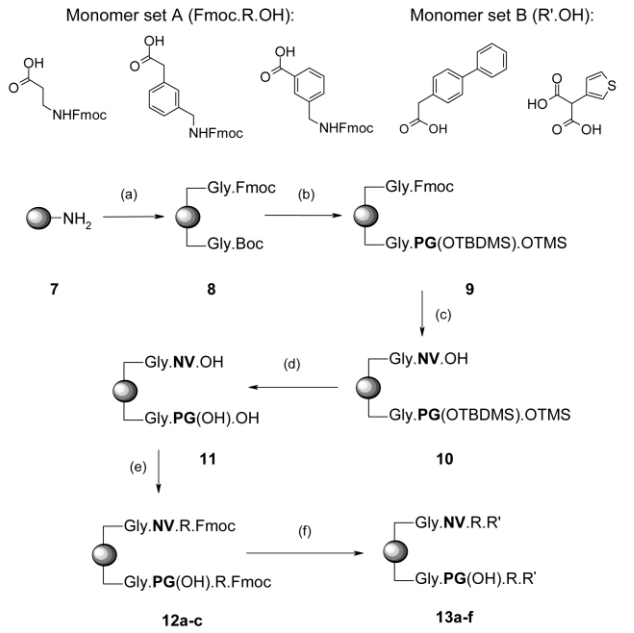
reliably achieved under the following conditions: (a) 355 nm, E = 500 μJ pulse^{-1}, t = 15 min; then (b) 300 nm, E = 130 μJ pulse^{-1}, t = 40 min.[11] Specifically, following irradiation under conditions (a), quantitative HPLC analysis (using a pre-determined calibration curve) of the supernatant solution established that 7.0 ± 0.7 nmol of substrate was isolated from each pool of 20 beads. Resubmission of the beads to conditions (a) resulted in no further substrate cleavage. However, changing to conditions (b) resulted in the isolation of a further 3.3 ± 0.3 nmol of substrate—but, again, no further cleavage was obtained upon continued irradiation. These results are consistent with exhaustive differential photolysis of a 2 : 1 [NV : PG] bifurcated resin.

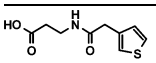
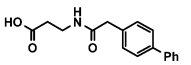
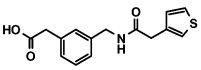
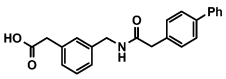
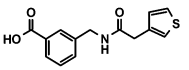
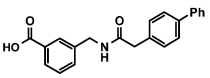
Having established general conditions for exhaustive photocleavage, we next prepared a small resin-bound compound array by a split synthesis (Scheme 1). Amino-Tentagel™ resin **7** was bifurcated in an approximately 1 : 1 ratio by coupling with an equimolar mixture of Fmoc.Gly and Boc.Gly to afford resin **8**. The *N*-protecting groups were removed sequentially and the NV and PG linkers incorporated to afford the resin **11** which was split into three equal parts and each coupled with a different Fmoc amino acid from the monomer set A to provide the resins **12a–c**. The Fmoc amino acids were chosen to include an aliphatic, benzylic and aromatic carboxylic acid. Following Fmoc deprotection, the resins were split into two batches, each of which was acylated with carboxylic acids from monomer set B to provide the resins **13a–f**.

Using an automated bead picker, 20 beads of each resin were placed into separate wells of a 96 well microtitre beadcup plate. Each resin pool was then suspended in DMSO (20 μl) and irradiated first at 355 nm (E = 500 μJ pulse^{-1}, t = 15 min) to release the material attached to the resin through the NV linker. The beadcups were drained by centrifugation, the beads washed once with DMSO, and the solutions were analysed by LC-MS. The wells were subsequently refilled with DMSO (20 μl) and subjected to a second laser irradiation (300 nm, E = 130 μJ pulse^{-1}, t = 40 min) to cleave material attached to the beads through the PG linker. The material released was then collected and relative peak areas determined by HPLC. Importantly, further irradiation of the resins at each stage did not yield additional material, thereby demonstrating that exhaustive photorelease had been achieved. By contrasting the relative peak areas obtained for the two different irradiations (Table 1) it is evident that the same amount of compound was released for both stages of the orthogonal release process for each member of the compound array **14–19**.



Scheme 1 *Reagents and conditions:* (a) (i) 30% (v/v) piperidine, DMF, (ii) (1 : 1) Fmoc.Gly/Boc.Gly, 1-hydroxybenzotriazole (HOBt), dicyclohexylcarbodiimide (DIC), DMF, 16 h; (b) (i) 2 × 50% (v/v) TFA/CH_2Cl_2, (ii) **2**, DIC, HOBt, *N*,*N*-diisopropylethylamine (DIPEA), DMF, 16 h; (c) (i) 30% (v/v) piperidine, DMF, (ii) **1**, excess HOBt, DIC, DMF, 5 h; (d) 1 M TBAF–THF, 16 h; (e) (i) split into 3; (ii) Fmoc.R.OH, DIC, 4-DMAP, CH_2Cl_2, DMF, 16 h; (f) (i) 30% (v/v) piperidine, DMF, (ii) split into 2, (iii) R′.OH, DIC, HOBt, DMF, 16 h.

Table 1 Cleavage ratios determined following differential photolysis of resins **13a–f** at 355 nm then 300 nm respectively

Cleavage product		AUC[a] $h\nu$ = 355 nm	AUC[a] $h\nu$ = 300 nm	Cleavage ratio[b] NV : PG
	**14**	384	380	1.0 : 1.0
	**15**	1273	1053	1.2 : 1.0
	**16**	467	421	1.1 : 1.0
	**17**	1283	1282	1.0 : 1.0
	**18**	985	963	1.0 : 1.0
	**19**	1300	1271	1.0 : 1.0

[a] Area under the curve by HPLC at 254 nm (average of n = 3 expts). [b] Compound purities were determined by LC-MS to be > 90% at 254 nm.

In summary, the photo-mediated controlled differential release of a compound array from solid phase resin can be achieved by sequential exhaustive photocleavage from a resin bifurcated with a combination of nitroveratryl and pivaloyl glycol photolinkers. In this way, similar amounts of material may be released from resin at each stage of the differential cleavage process prior to compound screening.

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9 The bead pools were pulsed at 10 Hz for 5 min each varying only the wavelength of the incident light (280–400 nm, 75 μJ pulse^{-1}) from well to well. An in-house developed laser photolysis system consisting of a continuum Nd : YAG pumped tuneable OPO laser directing the laser beam onto a beadcup plate mounted on a computer-controlled motorised X-Y stage was used in this study.
10 This ratio was consistent with a screening strategy under evaluation.
11 To minimise photo-degradation the laser was pulsed at 10 Hz with a typical pulse width of approximately 5 ns in all photolysis studies.