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Efficient Assembly of Peptomers on Continuous Surfaces

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Abstract: *The SPOT synthesis technique, originally developed for peptide synthesis on continuous surfaces, was adapted for high-throughput parallel solid phase synthesis of peptomers (peptide-peptoid-hybrids) using amino-functionalized cellulose membranes, a linker cleavable under dry conditions and modified synthesis protocols.*

SPOT synthesis on cellulose [1] is a highly effective method for the rapid and flexible preparation of high numbers of matrix-bound or cleavable peptides and subsequent screening [2,3]. This contribution describes the extension of the technique to the synthesis of spatially addressable peptomer librairies directly on continuous membranes. Initially, cellulose membranes (Whatman 50) were modified by treatment with epibromohydrin and 4,7,10-trioxa-1,13-tridecanediamine (Fig. 1), to provide homogeneous amino-functionalization [4]. Since synthesis on membranes via the SPOT technology is generally achieved without the use of any reactor vessel, final cleavage is preferably performed in the dry-state to give products adhesively bound to the membrane. The photo-labile linker system 4-[4-(1-Fmoc-aminoethyl)-2-methoxy-5-nitrophenoxyl]butanoic acid (Novabiochem), originally developed for cleavage of resin bound substances via UV irradiation of suspensions in various solvent systems [5], was tested for dry-state cleavage from membrane surfaces.

First, several activation methods were tested to achieve *N*-selective coupling of the linker onto the amino-membrane in the presence of cellulose hydroxyl functions (Fig. 1) [6]. Coupling with the highest selectivity (no ester formation) was obtained by activation via HATU and DIEA (1.0 eq. each) in NMP (0.5 M).

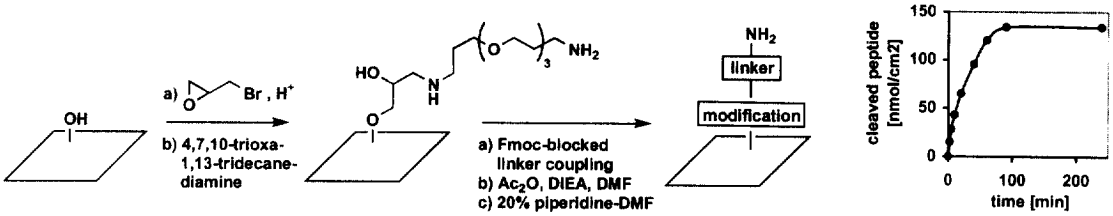

Figure 1: *Modification of a cellulose membrane, attachment of the linker and dry-state cleavage kinetics of a model peptide (LKY).*

To examine the cleavage kinetics of the photo-labile linker several dry spots containing a test peptide (H-LKY-NH_2; 140 nmol/cm^2 according to Fmoc determination) were exposed to UV light at 365 nm on a standard UV table (Vilber Lourmat TFX 20 LC; 7 mW/cm^2) at increasing irradiation times. The cleavage was shown to be quantitative after 90-100 min (Fig. 1).

The peptomer assembly via the SPOT technique on planar surfaces requires the efficient parallel incorporation of peptoidic and peptidic building blocks. Peptoid synthesis is usually performed by a two step procedure using bromoacetic acid for acylation and primary amines for nucleophilic substitution at the bromomethyl group (submonomer method) [7]. Bromoacetic acid 2,4-dinitrophenylester (1.0 M in NMP) was found to yield complete amino-acylation of peptidic or peptoidic building blocks with low ester formation at remaining cellulose hydroxyl functions and at peptoid units carrying unprotected OH groups (Fig. 2). The primary amines were

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used as 50% solutions in NMP or, depending on their solubility, as saturated solutions in NMP or water [8].
Fmoc deprotection following the introduction of an Fmoc amino acid needs to be carried out in parallel with treatment of an adjacent spot with an amine for the generation of a peptoid (Fig. 2). Piperidine was shown to be too volatile using SPOT synthesis conditions (displacement of bromine at adjacent bromoacetylated peptoid spots). The less volatile DBU was substituted for piperidine as deblocking agent. Experiments performed with solutions of 4% DBU in NMP resulted in complete Fmoc deprotection [8].
Subsequently, the effective acylation of the sterically hindered secondary amino function of a peptoidic building block with an Fmoc amino acid in the presence of free hydroxyl functions was required. The combination DIC/HOAt, reported to be well suited for peptomer synthesis on resins [9], gave only mediocre coupling yields using SPOT synthesis conditions on cellulose membranes. Therefore, several activation methods were examined for acylation of *N*-[2-(3,4-dimethoxyphenyl)ethyl]Gly-R(Pbf)-W(Boc)-NH_2 as model system with various Fmoc amino acids. It was found that highest acylation values were obtained via symmetric anhydrides and amino acid fluorides [8,10] at no significant esterification when used in the absence of base.

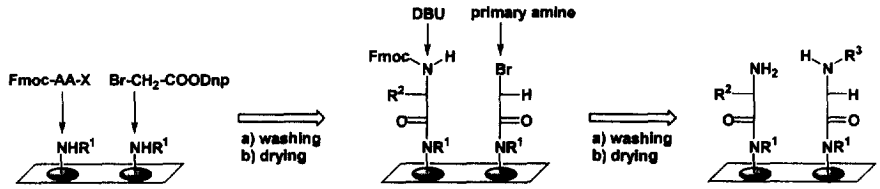

Figure 2: Reaction scheme for the parallel incorporation of peptidic and peptoidic building blocks in peptomer synthesis.

Finally, the optimized SPOT synthesis protocol developed was applied to a systematic peptide-peptoid-transformation of the peptide PPPPY, the consensus motif of the hYAP-WW-protein domain [11]. More than 350 peptomers were synthesized in sufficient yields and purities.

References and Notes

Abbreviations: DBU: 1,8-diazabicyclo[5,4,0]undec-7-ene; DIC: diisopropylcarbodiimide; DIEA: diisopropylethylamine; Dnp: 2,4-dinitrophenyl; Fmoc: 9-fluorenylmethyloxycarbonyl; HATU: *N*-[(dimethylamino)-1H-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAt: 1-hydroxy-7-azabenzotriazole; NMI: *N*-methylimidazole; NMP: *N*-methylpyrrolidone; PyAOP: 7-azabenzotriazol-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate; PyBOP: benzotriazol-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate; PyBroP: bromotris(pyrrolidino)phosphonium hexafluorophosphate; TBTU: *N*-[(1H-benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide.

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[4] A cellulose sheet (19x28 cm) is immersed in a 10% epibromohydrin/dioxane solution containing 1% perchloric acid (60% in water) for 1 hour, washed with methanol and incubated with 20% 4,7,10-trioxa-1,13-tridecanediamine in DMF for 1 hour. Subsequent washing of the membrane with 5 M sodium methanolate/methanol and methanol gave the amino-functionalized membrane (0.2 μmol/cm^2). Higher loadings (up to 1.5 μmol/cm^2) can be achieved with increased reaction times, use of neat 4,7,10-trioxa-1,13-tridecanediamine or increased temperature (80°C). In contrast to an earlier report (R. Volkmer-Engert et al., *Tetrahedron Lett.* **1997**, *8*, 1029) the present method yields in higher and adjustable loadings at markedly shorter reaction times.
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[6] TBTU, PyBOP and HATU (4-8 eq. relative to the amino-loading) were used for linker attachment. Preactivation was performed for 5 min with DIEA or NMI (1.0 or 2.0 eq.). DIC was used with 1.0 eq. NMI or without base. In all cases double couplings (2x15 min) were applied. Dimmed light is recommended due to the light sensitivity of the linker system.
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[8] Spot steps were performed three times (reaction time: 15 min each).
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[10] Fmoc amino acids: E(OtBu), F, G, I, K(Boc), M, P, Q(Trt), S(tBu), V, Y(tBu); activations: HATU, TBTU, PyAOP, PyBOP, PyBroP (1.0 eq., used with 1.0 eq. DIEA to suppress esterification), DIC/HOAt, DIC, fluorides. In contrast to earlier results (H. Wenschuh et al., J. Org. Chem. **1994**, 59, 3275) on coupling of sterically hindered α,α-dialkylated amino acids, anhydrides were more efficient (yield: 90-100%; Q(Trt): 65%) than fluorides (yield: 70-90%; Q(Trt): 30%) for the acylation of secondary amines.
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