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# A modular approach to the SPOT synthesis of 1,3,5-trisubstituted hydantoins on cellulose membranes

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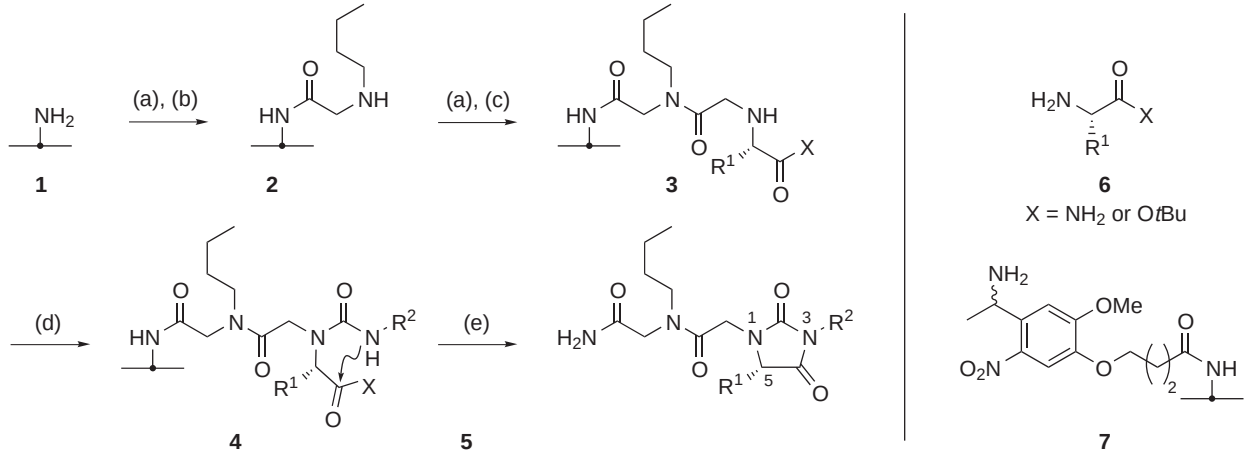
**Abstract**—The SPOT synthesis technique, originally developed for peptide synthesis on cellulose membranes, was successfully adapted for the modular, parallel synthesis of 1,3,5-trisubstituted hydantoins. The synthetic approach presented here is based on a modified sub-monomer peptoid synthesis protocol on cellulose membranes. © 2000 Published by Elsevier Science Ltd.

SPOT synthesis on cellulose membranes, introduced by R. Frank in 1992, has been proven to be a powerful method for the synthesis of peptide libraries.[1,2] Utilizing the excellent compatibility of the membrane support with solid phase binding assays the cellulose bound peptide arrays were shown to be well suited for the rapid investigation of molecular recognition events such as protein–protein interactions.[3] Recently, the SPOT




**Scheme 1.** SPOT synthesis of hydantoins on linker modified cellulose membranes. (a) Br-$CH_2$-COODnp (1 M in NMP, 2×15 min); (b) *n*-butylamine (5 M in NMP, 3×15 min); (c) **6** (1–5 M in $H_2O$ or NMP, 3×15 min); (d) *N*-benzylimidazole (3 M in NMP), then $R^2$-NCO (1 M in NMP, 2×15 min); (e) for Rink-linker: 95% TFA/$H_2O$, 60°C, 20 min, for photo-linker **7**: 95% TFA/$H_2O$, 60°C, 20 min, then *hv* (365 nm, 2×1 h). For $R^1$ and $R^2$, see Table 1.

*Keywords*: hydantoins; solid-phase synthesis; SPOT synthesis.

*Abbreviations:* AA, amino acid; $Ac_2O$, acetic anhydride; Bu, butyl; DIC, diisopropylcarbodiimide; DIEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; Dnp, 2,4-dinitrophenyl; ESI-MS, electrospray-ionization mass spectrometry; Fmoc, 9-fluorenyl-methyloxycarbonyl; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; Me, methyl; NMI, *N*-methylimidazole; NMP, *N*-methylpyrrolidone; Ph, phenyl.

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technique was extended to the assembly of peptidomimetics,[4] glycoconjugates,[5] and small organic molecules such as 1,3,5-triazines.[6] The present article reports on an additional application, namely the generation of hydantoins using coherent cellulose membranes as solid phase carriers.

Hydantoins were among the first and most extensively studied non-oligomeric structures combinatorially synthesized on solid supports.[7,8] In general hydantoins were assembled by stepwise synthesis of linear precursors and their subsequent cyclization. In most cases the heterocycles were selectively cleaved from the resin during the cyclization process releasing the desired products in high purities under acidic,[7,8a] basic,[8b] or neutral conditions.[8c] These methods are supplemented by procedures in which cyclization is performed prior to cleavage from the resin.[8d]

The present study describes our findings that synthesis protocols of oligo-*N*-alkyl glycines (peptoids)[9a] on planar membrane supports[4] can easily be modified to yield 1,3,5-trisubstituted hydantoins supporting ongoing efforts on the transformation of bioactive peptides to peptidomimetics as well as small heterocyclic compounds[10] by means of the SPOT synthesis concept.

In order to evaluate the generation of hydantoins from linear peptoid precursors the dipeptoids **3** were first synthesized on an amino derivatized cellulose membrane **1**,[4] which was modified with a suitable linker-system (Rink-linker[11] or photo-linker **7**,[12] Scheme 1). A first peptoid unit **2** was assembled by acylation of the linker-moiety with bromoacetic acid followed by nucleophilic substitution with *n*-butylamine at the bromomethyl group (sub-monomer method).[9] Applicability of amines for the generation of the second peptoid unit was limited to those enabling cyclization to the corresponding hydantoins. The amines required for this purpose were α-amino acid derivatives **6** bearing a carboxylic functionality suitable for hydantoin formation. While simple esters were widely used as linear precursors in hydantoin chemistry, amides or *tert*-butylesters have the advantage of being stable against amines at high concentrations necessary for the sub-monomer peptoid synthesis attempted in this study.[9] In order to overcome solubility problems of polar α-amino acid derivatives in commonly used solvents such as NMP or DMSO water could successfully be applied in this approach (in contrast to hydrophobic solid supports the cellulose surfaces described here enabled the use of reagents in aqueous solutions). The *N*-terminal amino functions of the dipeptoids **3** were treated with isocyanates $R^2$-NCO to form the ureas **4**. The reaction was catalyzed by the non-volatile *N*-benzylimidazole pipetted to the membrane prior to the isocyanate solution in order to limit degradation of the isocyanates compared to the application of a reagent/catalyst mixture. Cyclization was then performed under acidic conditions (95% TFA in $H_2O$) and shown to be complete after 10–20 min at 60°C (the reaction was incomplete at lower temperatures even after prolonged treatment,

**Table 1.** Hydantoin derivatives **5** obtained according to Scheme 1

| Entry | $R^1$ | X | (= H-AA-X) **6** | Solvent (conc.) for **6** | $R^2$ | Purity **5** (%)[a] |
|---|---|---|---|---|---|---|
| a |  | $NH_2$ | (= H-Gly-$NH_2$) | $H_2O$ (5.0 M)[b] | Ph | 81[c] |
| b |  | O*t*Bu | (= H-Gly-O*t*Bu) | $H_2O$ (2.5 M)[b] | Ph | 79[d] |
| c |  | $NH_2$ | (= H-Ala-$NH_2$) | $H_2O$ (5.0 M)[b] | Ph | 86[c] |
| d |  | $NH_2$ | (= H-Leu-$NH_2$) | $H_2O$ (5.0 M)[b] | Ph | 76[d] |
| e |  | O*t*Bu | (= H-Ile-O*t*Bu) | NMP (1.0 M) | Ph | 54[d] |
| f |  | $NH_2$ | (= H-Gly-$NH_2$) | $H_2O$ (5.0 M)[b] | *n*-Bu | 82[c] |
| g |  | $NH_2$ | (= H-Ala-$NH_2$) | $H_2O$ (5.0 M)[b] | *n*-Bu | 70[d] |

[a] HPLC (220 nm).
[b] 0.05% Tween® 20 added.
[c] Photo-linker **7**.
[d] Rink-linker.

data not shown). The ring-closure reaction was accompanied by cleavage of the products from the membrane when the Rink-linker was used (entries b, d, e, and g in Table 1) enabling the rapid optimization of reaction conditions.[13] However, a linker method which allows orthogonal cleavage of side chain protecting groups leaving the compounds covalently linked to the membrane is advantageous for solid phase screening approaches. Therefore, a photolytically cleavable linker system **7** was used enabling the cyclization while compounds remain covalently attached to the membrane.[12] Analysis was performed after irradiating the dried membrane at 365 nm and dissolving the released hydantoins **5** with buffer (entries a, c, and f in Table 1).[4]

Several hydantoins **5** were synthesized based on this synthesis scheme using amino acid amides (H-AA-$NH_2$) and *tert*-butyl esters (H-AA-O*t*Bu) differing in their sterical demand. Additionally phenyl- and *n*-butylisocyanate were used for the introduction of aryl and alkyl substituents in the 3-position (Table 1).[14] The corresponding hydantoins **5** were obtained with a purity of 54–86% (HPLC 220 nm).[15] The results showed that both amides and *tert*-butyl esters gave comparable results (entries a and b). In addition, *C*-substituted hydantoins were isolated with satisfying purities, if a branch in the α-position of the ring substituent was avoided (entries c and d versus e).[16]

In conclusion we demonstrated a new route to the solid phase synthesis (SPS) of hydantoins enabling combinatorial substitutions in the 1-, 3-, and 5-positions of the heterocycle. Whereas most of the procedures for the SPS of hydantoins rely on the presence of ester groups this potentially aminolysable functionality is avoided in the synthetic approach described here. The development of an efficient hydantoin synthesis protocol on planar membrane supports extends the scope of the SPOT synthesis technique to small organic molecules previously not readily accessible by this method.

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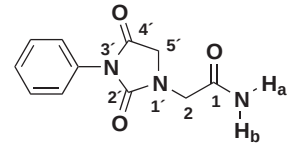
13. In order to avoid mixing of different compounds located on the same membrane the membrane bound linear hydantoin precursors **4** had to be punched out and transferred into separate reaction vessels prior to TFA treatment.
14. Experimental details for the synthesis of hydantoins **5**: The syntheses were performed on amino derivatized cellulose membranes[6] (loading: 0.5–0.9 μmol/$cm^2$) by repetitive pipetting of 2.0 μl aliquots of reagents (dissolved in NMP if not otherwise noted) to the membrane on marked positions (SPOTs; for general SPOT procedures see lit. 17). For Rink-linker attachment a 0.2 M solution of *N*-Fmoc-4-[amino-(2,4-dimethoxyphenyl)-methyl]-phenoxy acetic acid (Novabiochem GmbH, Bad Soden, Germany) pre-activated with DIC/pentafluorophenol (1.0 equiv. each, 30 min) was applied (2×15 min). The photolinker **7** was introduced as described previously.[6] Unchanged amino functions were capped in the course of a washing/acetylation/deprotection procedure [DMF (2×2 min), DMF/$Ac_2O$/DIEA 7:1:2 (v/v, 2×15 min), DMF (2×2 min), 20% piperidine in DMF (2×10 min), DMF (5×2 min), and MeOH (2×2 min)]. The linear precursors were synthesized in a stepwise fashion applying the following reagents: (1) Br-$CH_2$-ODnp (1.0 M, 2×15 min); (2) *n*-butylamine (5.0 M, 3×15 min); (3) Br-$CH_2$-ODnp (1.0 M, 2×15 min); (4) H-AA-$NH_2$ *or* H-AA-O*t*Bu according to Table 1 (3×15 min each); (5) *N*-benzylimidazole (3.0 M in DMF, 1×15 min) followed by the immediate addition of isocyanate (1.0 M, 2×15 min). Excesses of reagents (1), (3), and (5) were removed after the synthesis step by DMF (5×2 min) and MeOH (2×2 min); after reagents (2) and (4) DMF (4×2 min), MeOH (1×2 min), 0.5 M aq. NaOH (1×1

min), $H_2O$ (5×2 min), and MeOH (2×2 min) were used. Cyclization and cleavage was performed depending on the linker used: *Rink-linker*: treatment of a single SPOT (0.23 $cm^2$) with 70 μl of TFA (95% in $H_2O$) at 60°C and subsequent removal of the solvent *i. vac.* gave about 100–200 nmol of product; *photo-linker*: ureas **4** were cyclized by treatment of the membrane with TFA (95% in $H_2O$) at 60°C and products released after washing [MeOH and MeOH/$Et_3N$ 9:1 (2×2 min each)] by photolysis of the dried membrane on a UV table (Vilber Lourmat TFX 20 LC, 7 mW/$cm^2$, 365 nm, 60 min for each side of the membrane).

15. All compounds were analyzed by HPLC–MS (HP-1100 coupled to Finnigan LCQ). In addition, the hydantoin **8** was synthesized on a larger piece of Rink-linker derivatized membrane (25 $cm^2$) under comparable reaction conditions. In addition to standard LC–MS analysis, NMR and HR-MS data were recorded and found to be consistent with the desired structure: $^1H$ NMR (500 MHz, $d_6$-DMSO) $\delta = 3.95$ (s, 2H, 2-H), 4.14 (s, 2H, 5′-H), 7.27 (br-s, 1H, $CONH_2$), 7.34–7.51 (m, 5H, Ar-H), 7.65 (br-s, 1H, $CONH_2$); $^{13}C$ NMR (126 MHz, $d_6$-DMSO) $\delta = 45.0$ (C-5′), 50.7 (C-2), 126.5, 127.8, 128.8, and 132.2 (Ar-C), 155.8 (C-2′), 169.4, and 169.5 (C-1 and C-4′); FAB-HR-MS: calc.: 234.0879 ($M+H^+$), found: 234.0891.




**8**

16. Since a stereocenter was incorporated by the enantiomerically pure α-amino acid building blocks, possible racemization of the formed 5-substituted hydantoins needed to be studied: treatment of a 5-methyl hydantoin derived from alanine amide with $d_1$-TFA (95% in $D_2O$) at 60°C for 20 min (i.e. the conditions of cyclization) gave no incorporation of deuterium into the 5-position as determined by ESI-MS indicating that no significant racemization had occurred.[18]

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