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A Convenient Synthesis of Cyclic Peptides as Regioselectively Addressable Functionalized Templates (RAFT)

P. Dumy*, I. M. Eggleston, S. Cervigni, U. Sila, X. Sun and M. Mutter.

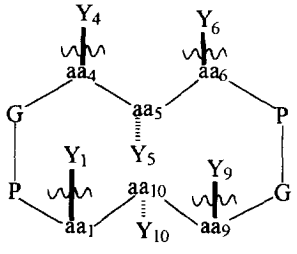
Institute of Organic Chemistry, University of Lausanne, BCH-Dorigny, CH-1015 Lausanne, Switzerland

Abstract: Cyclic decapeptides containing up to four orthogonally protected lysine residues have been efficiently prepared by a combined solid phase and solution strategy. These cyclic peptides represent new Regioselectively Addressable Functionalized Templates (RAFT), which are suitable for use in protein de novo design according to the TASP concept.

Selectively addressable topological templates represent a key feature in the de novo design of proteins using the TASP concept (Template Assembled Synthetic Proteins)[1]. In this approach, a peptidic template is used as a structural motif upon which amphiphilic peptide secondary structure units are assembled and thus directed into given folding topologies.

Recent studies from our laboratory in the area of TASP design[2,3] have concentrated on the use of lysine-containing decapeptides of the general formula shown in Figure 1. These provide conformationally well-defined templates based on two linked type II β-turns. Such templates typically contained only one kind of lysine side-chain protection, for in general, the preparation of cyclic peptides with two or more orthogonal protecting groups is rather complicated, especially by solution methods[2]. The synthesis of more elaborate TASP molecules however, now requires cyclic peptide templates with several selectively addressable sites and hence several orthogonally protected side-chains. For this reason, we have further refined our synthetic approach to topological templates of this kind by utilizing the greater flexibility of solid-phase methods to rapidly obtain the appropriate multiple-orthogonally protected linear precursors. These peptides may then be cyclized in solution with unusually high levels of efficiency and reproducibility[4]. The combined solid-phase and solution strategy which we present here allows access to template molecules containing an unprecedented number of orthogonal protecting groups or selectively addressable sites. We term such molecules **R**egioselectively **A**ddressable **F**unctionalized **T**emplates (**RAFT**).

Fig. 1. *General features of RAFT molecules*:



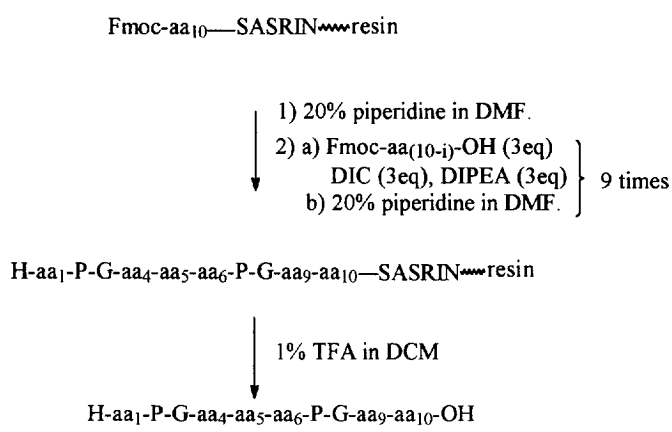
1b-4b

RAFT molecules represent peptidic templates which contain trifunctional amino-acids (aa_i) whose side-chains may be selectively addressed by virtue of orthogonal protection techniques, or a unique chemical reactivity. Table 1 shows examples of RAFT containing regioselectively addressable lysines, bearing orthogonal protecting groups, Y_i, on the side-chain amino group.

G=Gly; P=Pro; K=Lys; A=Ala; see Table 1

Our new approach and its scope is illustrated by the preparation of **RAFT** molecules **1b-4b**. Linear peptides **1a-4a** were assembled using standard Fmoc solid-phase chemistry with a highly acid-labile linker unit, the Sasrin® handle[5] (Scheme 1). Such a combination of very mild coupling and deprotection protocols[6,7] thus provides the flexibility needed in order to incorporate a variety of side-chain protecting groups, cleavable by acid (Boc), base (Fmoc), Pd(II) (Aloc[8]), or nucleophiles (Dde[9]). This represents a level of orthogonality seldom available by other synthetic strategies, with **3a** being probably the first example of a simple peptide which contains four identical amino-acids that are orthogonally protected and thus selectively addressable.

Scheme 1.



1a-4a

1a-4a were obtained[6] in excellent yields (around 80%) and high purity, as judged by HPLC, and were characterized by FABMS[10]. Their cyclizations were then effected with PyBOP[11] in DMF[6] at high dilution. Despite the high dilution, the reactions proceeded remarkably rapidly and were generally complete within 30 minutes, even with only 1.1 equivalents of PyBOP. This is in direct contrast to typical solution cyclizations where excess reagents and long reaction times are usually required[2,12]. For our systems, the acceleration is probably due to the existence of preferred quasi-cyclic conformations in the linear templates[13] when the point of cyclization is as chosen here (**head** aa_1 to **tail** aa_{10}), and as such it is an additional advantage of our new synthetic strategy. The yields were moreover, consistently good and compare very favourably with those recently reported for methods of cyclic peptide synthesis involving cyclization on the solid support itself[14]. Finally, the pure cyclic templates could be isolated by simple precipitation, without any need for HPLC purification.

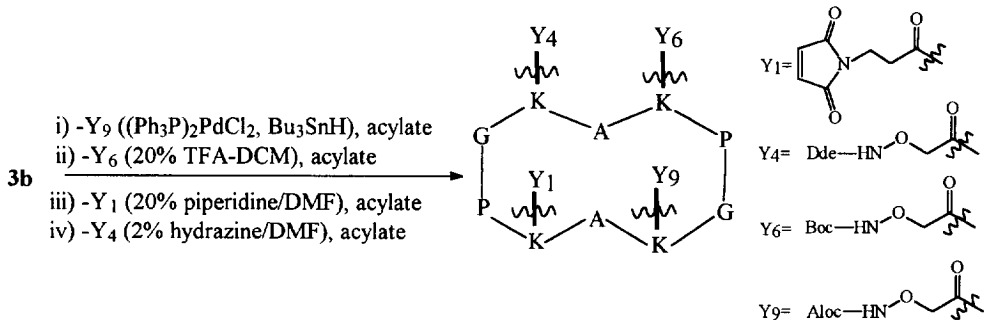
Once again, the integrity of the products was confirmed by HPLC and FABMS[15], and additionally by high field NMR spectroscopy[16]. The convenience and the generality of the approach therefore make it especially suitable for the preparation of gram quantities of **RAFT**, including those in which the basic template structure is modified by, for example, the substitution of other β-turn inducing moieties[17].

Table 1 *Cyclization yields for the synthesis of **RAFT** molecules.*

aa_i (Y_i)	1	4	5	6	9	10	%
1b	K(Boc)	K(Aloc)	A	K(Boc)	K(Aloc)	A	82
2b	K(Boc)	K(Boc)	A	K(Boc)	K(Aloc)	A	63
3b	K(Fmoc)	K(Dde)	A	K(Boc)	K(Aloc)	A	52
4b	K(Boc)	K(Boc)	K(Aloc)	K(Boc)	K(Boc)	K(Aloc)	73

Template molecules such as **1b-4b** now offer the possibility of conversion to novel **RAFT** which are suitable for the chemoselective ligation of unprotected peptide blocks - a means of overcoming the poor solubility of fully protected peptide derivatives in TASP synthesis[18]. In the case of template **3b**, this has already been achieved, by carefully applying documented orthogonal deprotection methods to selectively reveal one unique amino group at a time. This is then acylated[19] with an orthogonally protected aminoxy derivative or a maleimide derivative to create a new regioselectively addressable site[17,20], (see Figure 2). By respecting the proper order of deprotection-acylation (Y_9, Y_6, Y_1, then Y_4) it was possible to maintain the original high level of orthogonality throughout the whole sequence.

Fig. 2.*Preparation of **RAFT** containing novel regioselectively addressable functionnalities.*



Further possibilities also now under study include the preparation of novel TASP from **RAFT** such as **4b**, wherein orthogonal protection ultimately permits "effector" and "receptor" properties to be differentiated on opposite faces of the template, and the use of **RAFT** for creating structural motif-based libraries[3,21].

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4 Kopple, K.D. *J. Pharm. Sci.* **1972**, *61*, 1345-1356.
5. Mergler, M.; Tanner, R.; Gosteli, J.; Gregg, P. *Tetrahedron Lett.* **1988**, *29*, 4005-4008.
6 A typical procedure was as follows: Using N^{α}-Fmoc-Ala-Sasrin® resin (1.92g, 1.30 mmol), the linear protected decapeptide was assembled using N^{α}-Fmoc-protected amino acids (3.90 mmol), preactivated using HOBt (0.53g, 3.90 mmoles) and N,N-diisopropylcarbodiimide (DIC) (0.81g. 3.90 mmol) in DMF during 15 min, with coupling in the presence of diisopropylethylamine (DIPEA) (0.67ml, 3.90 mmol) in DMF for 40 min. The completeness of each coupling was confirmed by the Kaiser test[22]. The N^{α}-Fmoc-protecting groups were removed by treatment with piperidine (20% v/v in DMF, 3 min, then 15 min cycle); the completeness of each deprotection being verified by the UV absorption of the piperidine washings at 302 nm[23]. The protected peptide was then cleaved from the resin with 1% TFA in DCM (15 ml, 6 cycles of 10 min) and was neutralized with 1% pyridine in DCM (6x15 ml). The solvent was removed under reduced pressure to give a gum from which the linear decapeptide **1a** (1.63g, 83%) was obtained by precipitation from DCM-diethyl ether. A solution of **1a** (0.73g, 0.5 mmol) and PyBOP (0.39g, 0.75 mmol) in DMF (800 ml) was treated at room temperature with a solution of DIPEA (0.30 ml, 1.75 mmol) in DMF (5ml), added over 10 min. The cyclization was complete after an additional 20 min, as determined by analytical reversed phase HPLC, and the solution was concentrated under reduced pressure. The residue was dissolved in DCM and diethyl ether was added to give the pure cyclic decapeptide **1b** (0.55g, 82%) as a white solid.
7. In the case of template **3b**, the fourth orthogonal protecting group was incorporated by using Trt-Lys(Fmoc)OH in the last coupling cycle.
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10. Selected data, **1a** : FABMS (matrix: thioglycerol) $[M+H]^+$=1350, $[M\text{-}Boc+2]^+$=1250, $[M\text{-}2xBoc+3]^+$=1150.
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15. Selected data, **1b** : FABMS (matrix: nitrobenzyl alkohol) $[M+Na]^+$=1354, $[M+H]^+$=1332, $[M\text{-}Boc+2]^+$=1232, $[M\text{-}2xBoc+3]^+$=1132.
16. The detailed solution conformations of **1b** and **3b** were elucided by 600MHz DQF-COSY, TOCSY and NOESY experiments. Dumy, P.; Eggleston I. M.; Esposito G.; Mutter, M. manuscript in preparation.
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19. A typical procedure for deprotection and acylation was as follows: A solution of the template **3b** (150mg, 0.095mmol) and $(Ph_3P)_2PdCl_2$ (2-3mg) in DCM/2% AcOH (2ml) was treated with nBu_3SnH (25µl, 0.09mmol) added in two portions over 5 min. When the reaction was judged complete by HPLC, the orange solution was filtered and was concentrated *in vacuo*, and diethyl ether was added. The precipitate thus obtained was isolated by centrifugation and the ether was decanted. The precipitate was washed several times with ether, and was finally dried *in vacuo* to give the desAloc derivative as a white powder (129mg, 91%). ESMS: 1452=M+H. A mixture of Aloc-NH-O-CH_2CO_2Su (35mg, 0.17mmol) and the desAloc-template (129mg, 0.087mmol) in DMF (2ml) was treated with DIEA (14µl, 0.087mmol). After 15 min, HPLC showed the coupling to be complete, and so the solvent was removed *in vacuo* and the residue was precipitated from DCM/diethyl ether. The heterogeneous mixture was centrifuged and the supernatent was decanted, then the solvent was evaporated to give a white powder. The powder was dissolved in water, and the solution was filtered and lyophilized to give the modified **RAFT** (140mg, 100%). ESMS (after Boc cleavage): 753.5=(M+2H)/2.
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