Report

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String Synthesis. A Spatially Addressable Split Procedure

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In the split-mix (originally called portioning-mixing) method introduced by Furka and his colleagues[1] in 1988 to prepare combinatorial libraries, each cycle of the solid-phase synthesis[2] was replaced by the following operations: (i) portioning of the solid support into equal samples, (ii) coupling a different monomer to each sample, and finally, (iii) mixing the samples. These operations were repeated until the desired length of oligomers was achieved.

The synthesis is very efficient, makes it possible to easily prepare millions of new substances in a short time, and produces individual compounds on each bead of the solid support. The quantity of the formed substances in the microscopic beads, however, is very low, and the identity of the products is unknown until determined in a separate deconvolution procedure. Using the parallel synthetic methods, known compounds can be prepared in multi-milligram quantities. The synthesis, even in its automated form, is very slow and expensive. For this reason efforts have been made to combine the high efficiency of the split synthesis with the advantages of the parallel method. In the methods developed to solve the problem, the microscopic individual beads of the solid support are replaced either by their macroscopic assemblies enclosed in permeable capsules[3,4] or by small, solid plates grafted and functionalized on their surfaces.[5] Both kinds of support units are labeled. Besides the beads, the capsules contain an electronic chip which stores an electronic code, and the plates carry a two-dimensional bar code etched by laser onto the ceramic support. In the procedure commercialized by IRORI,[6] the capsules are reacted in groups and after each synthetic step they are pooled and fed into an automatic machine for sorting. In the sorting process, first the electronic codes are determined, and then the capsules, one at a time, are automatically delivered into their destination reaction vessels. Sorting of 10 000 capsules takes about 10 h. A method replacing the individual support units by unlabeled regions of a thread wrapped around a cylinder has also been described.[7]

In this paper, a new spatially addressable split method is suggested which makes coding of the macroscopic support units unnecessary and, at the same time, speeds up sorting.

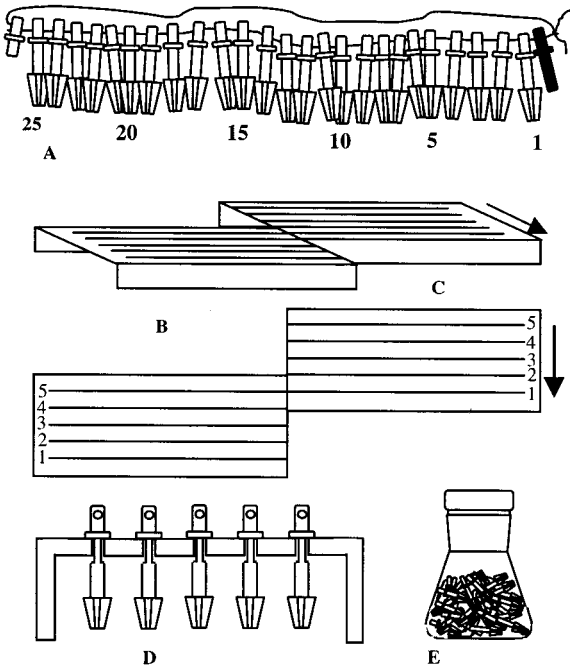

Figure 1. Stringed crowns and the manual sorter: (A) crowns on string, (B, C) side and top view of the manual sorting device, (B) source tray, (C) destination tray, (D) crowns in the slots of the sorter, (E) stringed crowns in flask.

According to theoretical considerations, a split synthesis using unlabeled macroscopic support units can be realized if (i) the unlabeled support units are arranged into spatially ordered groups and the relative spatial arrangement of the units is maintained during the chemical reactions, (ii) the support units are redistributed between the reaction steps according to a predetermined pattern permitted by the redistribution rule of the split synthesis, and (iii) the sorting process is simulated by a computer that can trace the synthetic history of each support unit.

To exemplify such a synthesis, a 125-member tripeptide library has been prepared using Chiron crowns[8] as solid support units and a simple manual device for sorting (Figure 1). Theoretically, there are different possibilities for formation of spatially ordered groups of support units. We used the simplest approach: stringing.[9,10] The crowns were attached to their somewhat reshaped stems (D). The holes in the stems made it possible to string the crowns on a polyethylene fishing line. One end (the tail) of the string was labeled by a shortened stem. The heads of the strings (from where the positions of the crowns were counted) were marked by differently colored full stems (A). Black, green, blue, yellow, and red colors corresponded to string numbers 1, 2, 3, 4, and 5, respectively. The manual sorting device consisted of two identical trays. One of them (B) was used as a source tray the other one (C) as a destination tray. Both trays had five parallel slots for holding the crowns. The crowns were hanging in the slots as shown in D.

In order to start the string synthesis, five strings were formed, each containing 25 crowns. Each string was placed

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Table 1. Amino Acids Used in the Three Coupling Steps

step	string 1 black	string 2 green	string 3 blue	string 4 yellow	string 5 red
1	isoleucine, I	phenylalanine, F	leucine, L	valine, V	glycine, G
2	glutamic acid, E	phenylalanine, F	tryptophane, W	tyrosine, Y	serine, S
3	glutamic acid, E	phenylalanine, F	tryptophane, W	tyrosine, Y	serine, S

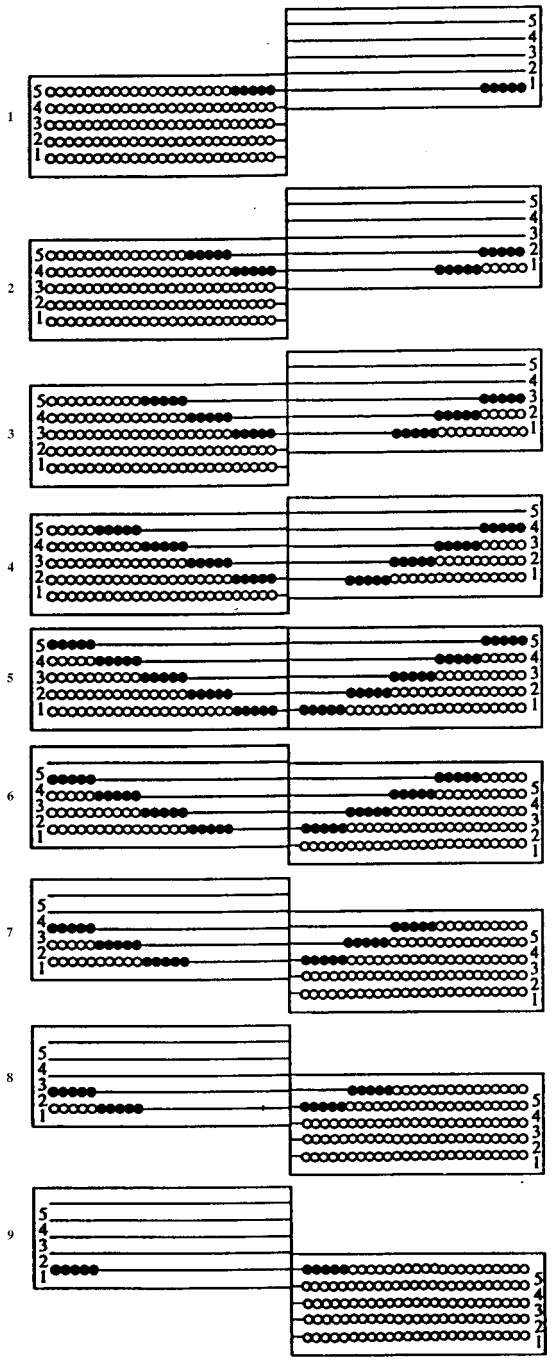

Figure 2. First sorting in nine stages.

into a flask (Figure 1D) and then coupled with the amino acids listed in the first row of Table 1. After washing and drying, the crowns were transferred (still in stringed form) into the slots of the source tray, and then the strings were removed. The crowns of strings 1, 2, 3, 4, and 5 were placed into slots 1, 2, 3, 4, and 5, respectively. The heads of the strings were oriented toward the numbered end of the source slots. Sorting was done by moving the destination tray of the sorting device from the start position (stage 1 in Figure 2) in eight stages into the end position (stage 9) and pushing crowns from the slots of the source tray into the slots of the destination tray. To ensure formation of all possible structural

Table 2. Content of String 1 in Different Stages of the Synthesis[a]

Position	C1	C2	C3	C4	C5
1	I-*	I-*	EI-*	EI-*	EEI-*
2	I-*	I-*	EI-*	FI-*	EFI-*
3	I-*	I-*	EI-*	WI-*	EWI-*
4	I-*	I-*	EI-*	YI-*	EYI-*
5	I-*	I-*	EI-*	SI-*	ESI-*
6	I-*	F-*	EF-*	EF-*	EEF-*
7	I-*	F-*	EF-*	FF-*	EFF-*
8	I-*	F-*	EF-*	WF-*	EWF-*
9	I-*	F-*	EF-*	YF-*	EYF-*
10	I-*	F-*	EF-*	SF-*	ESF-*
11	I-*	L-*	EL-*	EL-*	EEL-*
12	I-*	L-*	EL-*	FL-*	EFL-*
13	I-*	L-*	EL-*	WL-*	EWL-*
14	I-*	L-*	EL-*	YL-*	EYL-*
15	I-*	L-*	EL-*	SL-*	ESL-*
16	I-*	V-*	EV-*	EV-*	EEV-*
17	I-*	V-*	EV-*	FV-*	EFV-*
18	I-*	V-*	EV-*	WV-*	EWV-*
19	I-*	V-*	EV-*	YV-*	EYV-*
20	I-*	V-*	EV-*	SV-*	ESV-*
21	I-*	G-*	EG-*	EG-*	EEG-*
22	I-*	G-*	EG-*	FG-*	EFG-*
23	I-*	G-*	EG-*	WG-*	EWG-*
24	I-*	G-*	EG-*	YG-*	EYG-*
25	I-*	G-*	EG-*	SG-*	ESG-*

[a] Stages of synthesis: (C1) after first coupling; (C2) after first sorting; (C3) after second coupling; (C4) after second sorting; (C5) after third coupling.

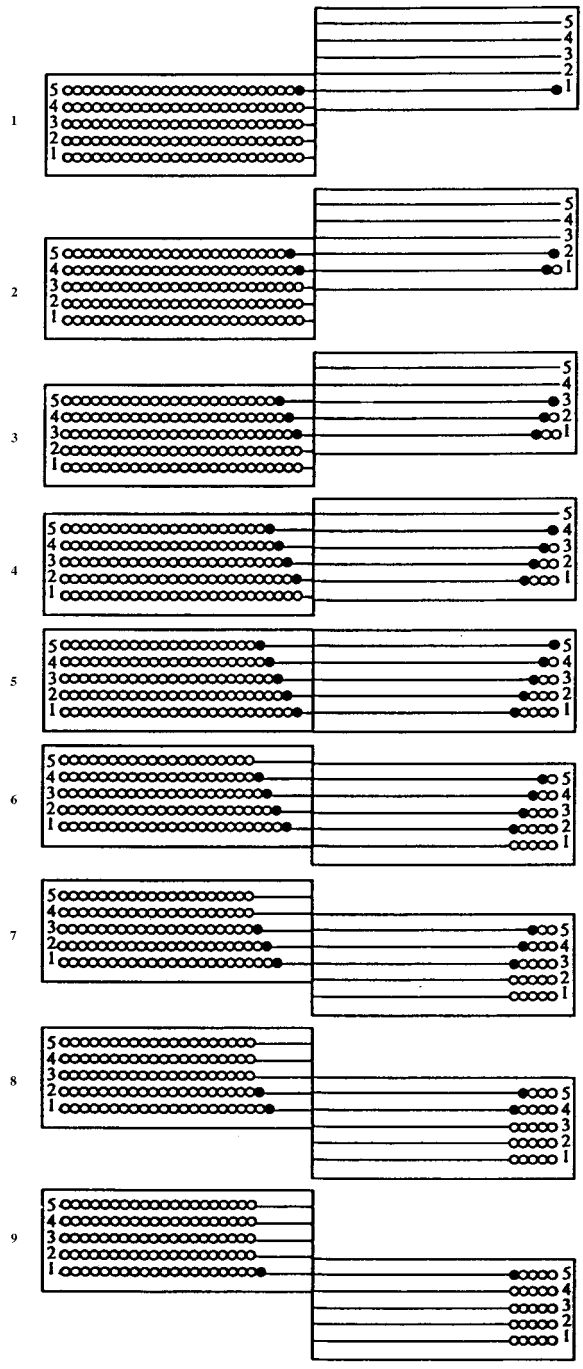

Figure 3. First round of second sorting in nine stages.

Table 3. Position of Tripeptides on the Strings[a]

Position	String1 Black	String2 Green	String3 Blue	String4 Yellow	String5 Red
1	EEI-*	FEI-*	WEI-*	YEI-*	SEI-*
2	EFI-*	FFI-*	WFI-*	YFI-*	SFI-*
3	EWI-*	FWI-*	WWI-*	YWI-*	SWI-*
4	EYI-*	FYI-*	WYI-*	YYI-*	SYI-*
5	ESI-*	FSI-*	WSI-*	YSI-*	SSI-*
6	EEF-*	FEF-*	WEF-*	YEF-*	SEF-*
7	EFF-*	FFF-*	WFF-*	YFF-*	SFF-*
8	EWF-*	FWF-*	WWF-*	YWF-*	SWF-*
9	EYF-*	FYF-*	WYF-*	YYF-*	SYF-*
10	ESF-*	FSF-*	WSF-*	YSF-*	SSF-*
11	EEL-*	FEL-*	WEL-*	YEL-*	SEL-*
12	EFL-*	FFL-*	WFL-*	YFL-*	SFL-*
13	EWL-*	FWL-*	WWL-*	YWL-*	SWL-*
14	EYL-*	FYL-*	WYL-*	YYL-*	SYL-*
15	ESL-*	FSL-*	WSL-*	YSL-*	SSL-*
16	EEV-*	FEV-*	WEV-*	YEV-*	SEV-*
17	EFV-*	FFV-*	WFV-*	YFV-*	SFV-*
18	EWV-*	FWV-*	WWV-*	YWV-*	SWV-*
19	EYV-*	FYV-*	WYV-*	YYV-*	SYV-*
20	ESV-*	FSV-*	WSV-*	YSV-*	SSV-*
21	EEG-*	FEG-*	WEG-*	YEG-*	SEG-*
22	EFG-*	FFG-*	WFG-*	YFG-*	SFG-*
23	EWG-*	FWG-*	WWG-*	YWG-*	SWG-*
24	EYG-*	FYG-*	WYG-*	YYG-*	SYG-*
25	ESG-*	FSG-*	WSG-*	YSG-*	SSG-*

[a] The sequences in boxes were randomly selected to compare them with independently prepared samples.

combinations in the synthesis, sorting had to follow the redistribution rule of the split synthesis: the support units of a string that carried the same compound had to be evenly distributed among the strings of the next reaction step.

First Sorting. After the first coupling step, each of the 25 crowns of a string carried the same attached amino acid, one of those listed in the first row of Table 1 (see, for example, string 1 in Table 2, column C1). These 25 crowns were distributed into five destination strings in blocks of five pieces. The first sorting which was carried out in nine stages is demonstrated in Figure 2 where the crowns are indicated by small circles. In each stage, position of the destination (right) tray relative to the source (left) one is different. In stages 1, 2, 3, 4, 5, 6, 7, 8, and 9, the number of slots in the source tray that are aligned with a slot in the destination piece is 1, 2, 3, 4, 5, 4, 3, 2, and 1, respectively. All these aligned slots are utilized for simultaneous transfers. Since the crowns were transferred from each slot in blocks of five pieces, in positions 1, 2, 3, 4, 5, 6, 7, 8, and 9 the number of crowns pushed simultaneously from the source piece into the destination one was 5, 10, 15, 20, 25, 20, 15, 10, and 5, respectively. In Figures 2 and 3, the source and destination positions of the transferred crowns are marked by black circles. The result of sorting can be exemplified by a single string (e.g., string 1 in column C2 of Table 2) since after sorting the spatial arrangement of the products on all destination strings was exactly the same. After the first sorting, the crowns, while still hanging in the slots of the destination tray, were stringed. The heads of the strings were considered oriented toward the unnumbered ends of the

destination slots. The stringed crowns were removed from the slots then submitted to the second coupling using the amino acids listed in row 2 of Table 1. The content of string 1 after the second coupling is shown in Table 2, column C3. The other strings differed from string 1 only by the N-terminal amino acid of the formed dipeptides.

Second Sorting. After the second coupling, the destination strings of first sorting became the source strings of second sorting. As exemplified by string 1 in Table 2, these strings consisted of five groups of crowns. The five crowns of each group carried the same dipeptide. These crowns had to be distributed among five destination strings which required a one by one transfer from each string. The first round of second sorting is demonstrated in Figure 3. Since only one crown was delivered at a time from each slot, in the nine stages shown in the figure, only 25 crowns were transferred ($1 + 2 + 3 + 4 + 5 + 4 + 3 + 2 + 1$). The nine stages had to be repeated four additional times to finish sorting. Thus the transfer of the 125 crowns needed altogether 45 stages. The result of sorting is again exemplified with the content of string 1 shown in Table 2, column C4, since each one of the five strings was the same. In every string, however, the 25 dipeptide sequences were different. After the second sorting, the crowns were stringed again and submitted to the third coupling with the amino acids of the row 3 of Table 1. The tripeptides formed on the crowns of string 1 are listed in Table 2, column C5.

The software[11] developed to simulate the full process predicted the positions of the final tripeptides as shown in Table 3. To prove the prediction, five sequences were randomly selected, one from each string (see the sequences in boxes in Table 3). These sequences were independently synthesized on crowns and, after cleavage, their properties were compared with those of the five tripeptides formed on the strings. Comparison of the HPLC chromatograms proved without any doubt the identity of the five pairs of tripeptides.

Our experiments show that a split synthesis using macroscopic solid support units can be realized without attaching to the support units any kind of label. The products can unambiguously be identified so no deconvolution is needed in screening of libraries produced by the method. In addition to peptide libraries, the string synthesis is expected to work in preparation of different organic libraries, and different kinds of solid support units can be used including resin containing capsules. The manual sorting device is very simple and can be easily built. Such a device could sort up to around 1000 units. The "pattern sorting" is an essential part of the new procedure. Different sorting devices can be constructed and different sorting patterns can be used that comply with the redistribution rule of split synthesis. According to our estimations, pattern sorting opens a possibility to construct a very fast automatic sorting machine.

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Supporting Information Available. MS results of five library components and HPLC chromatograms of the same five library members and five separately synthesized tripeptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The software was written in Visual Basic language and could trace all changes in the content and the spatial position of the crowns in the whole synthetic process. For more about software visit http://szerves.chem.elte.hu/Furka.

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