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An Efficient Synthesis and Screening of Osteostatin analogues by Using Pooling Strategy

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Abstract: An efficient synthesis and screening of twenty one osteostatin(OSN) analogues were achieved by using pooling strategy. Three analogues were found to retain almost the same activity of inhibiting bone resorption in rat as that of OSN. Therefore, it is possible to develop partially modified OSN surrogate with higher enzymic stability.

Keywords: Structure modification, pooling strategy, OSN analogues, osteoporosis.

Resistance to enzymatic hydrolysis is very important for the development of endogenic peptide-based drugs. Previous studies[1,2] have revealed it is possible that partially structure modified analogues could not only retain their biological activity but prevent metabolism of adjacent as well as remote peptide bonds also. However, to synthesize and screen large number of analogues is always a laborious task. The idea of combinatorial chemistry such as the Split-Mix method[3] , a major strategy for high throughput synthesis, is very suitable for preparing large sized libraries of chemicals instead of conventional synthesis and screening in one by one manner.

It was reported that OSN,a fragment of parathyroid hormone-related protein(PTHrP) 107-111, was a potent inhibitor of osteoclastic bone resorption in rat[4,5] . In the present study, we proposed a pooling strategy rather than split-mix method aimed at preparing smaller sized libraries with convenient operation of re-assembling the concerned OSN surrogates individually for iterative screening. The operation of pooling strategy is schemed briefly in **Figure 1** and **2**.

In order to afford the building blocks needed to re-assemble the concerned compounds for iterative screening, the intermediates produced after each coupling step should be pooled only in partial amount. The remainder of every intermediate was saved respectively for possible use in partial synthesis individually. Based on pooling strategy twenty-one OSN analogues (**Figure 3**) were synthesized manually in Boc-chemistry on the BNR support[6], and their structures were confirmed by amino acids analysis. Bioassay was carried out in rats treated with OSN analogues and OP pathogenic agent retinoic acid simultaneously for eleven days. The bioactivity of these synthetic products was evaluated by the comprehensive analysis from the observed functions such as body weight, fractures, femoral diameter ,

number of integrate femurs , femoral dry weight and crippling behavior. Based on these analysis, three analogues **g.i.r**(see **Figure 3**) showed almost the same activity as that of OSN.

Figure 1 Pooling operation in solid-phase synthesis

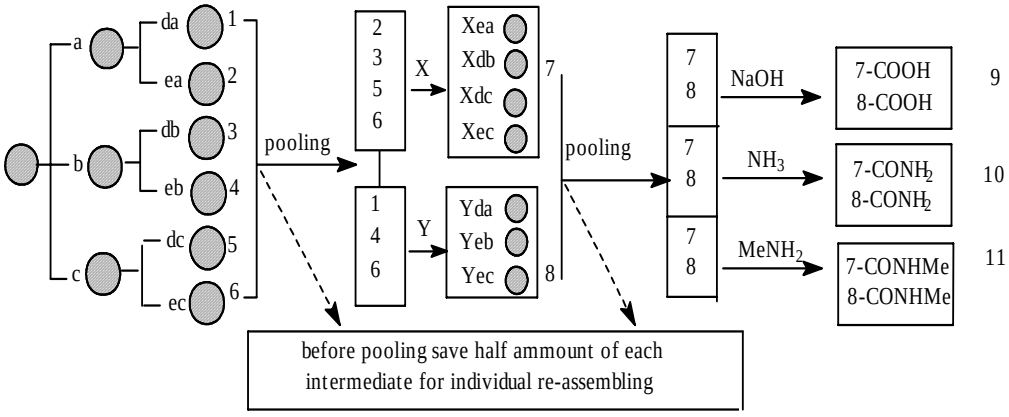


Figure 2 Iterative screening with pooling procedure

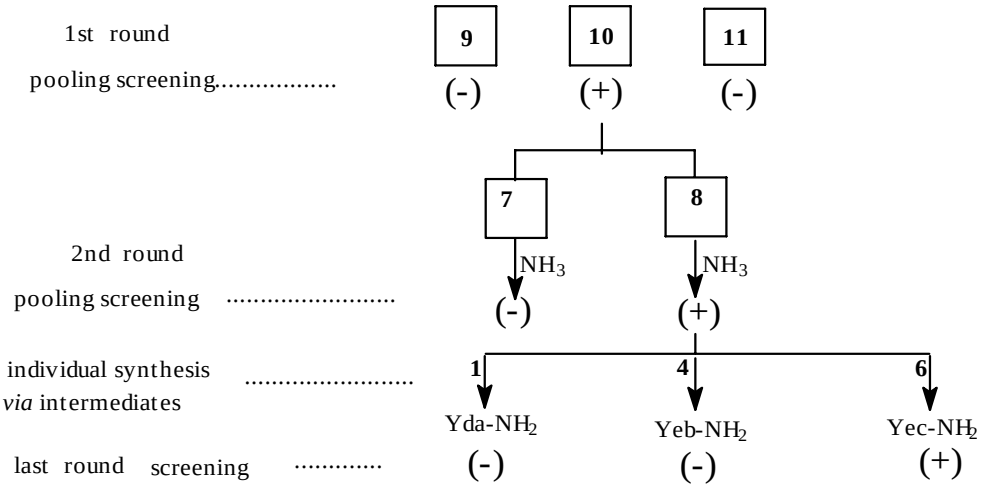
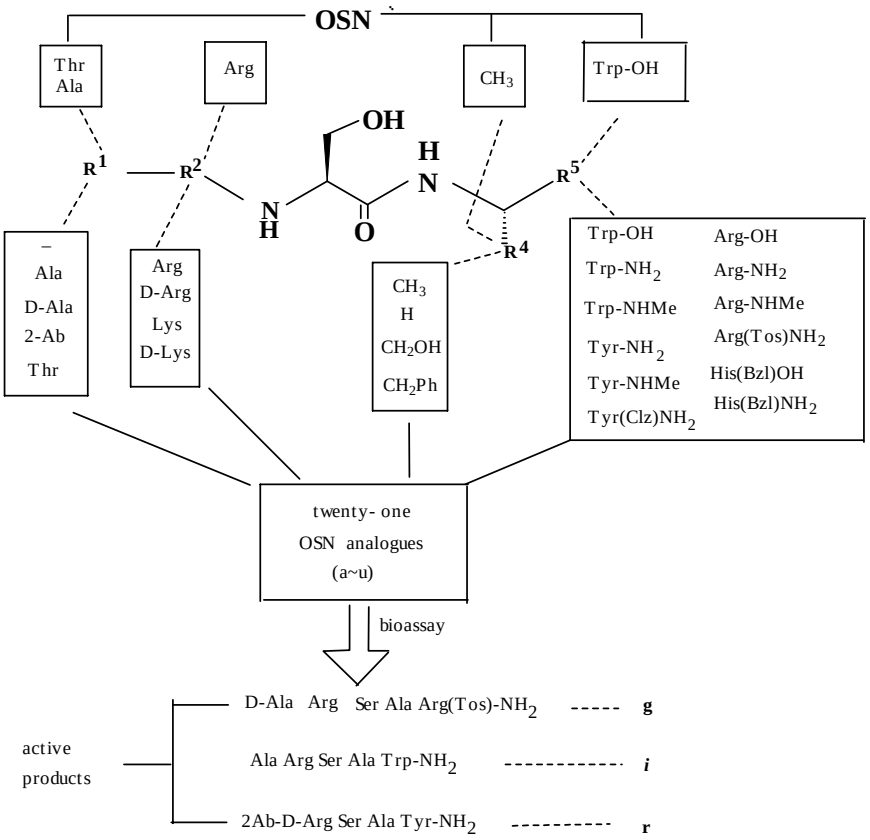


Figure 3 Building blocks in OSN surrogates



Summarizing the above results, we have found that : 1) the structure in the middle region (residue 3,4) of OSN molecule is highly conservative; 2) Arg (L- or D- form) at position 2 is necessary; 3) N terminal could not be deleted, but can be changed with other amino acids even including unnatural one, such as 2Ab; 4) it is imperative to keep aromatic moiety in the C terminal structure; 5) compared with split-mix method, pooling strategy is an economical way to synthesize and screen smaller sized chemical libraries.

In conclusion, the results from this study spurred us to make the refinement of introducing other unnatural building blocks to N or C terminal in OSN molecule, intending to develop some ideal OSN surrogates with higher bioavailability as the candidate for anti-osteoporosis drug.

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