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Combinatorial Solid-Phase Synthesis of Balanol Analogues

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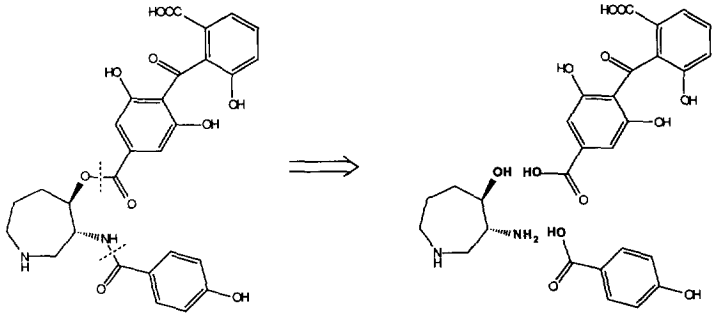
Abstract: The natural product balanol has served as a template for the design and synthesis of a combinatorial library using solid-phase chemistry. Using a retrosynthetic analysis, the structural analogues have been assembled from three relatively accessible building blocks. The solid-phase chemistry including MSNT-mediated esterification of both support-bound alcohols and carboxylic acids has been implemented successfully. Copyright © 1996 Elsevier Science Ltd

Protein kinase C (PKC) belongs to a family of serine/threonine specific kinases which are involved in a variety of processes including signal transduction, cell proliferation and cell differentiation. Agents that inhibit PKC may have wide ranging therapeutic potential, since activated PKC has been implicated in numerous disease processes. These include some wide-spread and severe diseases such as cancer, inflammation, cardiovascular dysfunctions, diabetic complications, asthma, central nervous system disorders and HIV infection.[1]



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Retrosynthetic analysis of balanol, **1**.

Scheme 1

Balanol (**1**) is a fungal metabolite, which has attracted significant attention because it possesses high PKC inhibiting activity. Furthermore, balanol has a relatively favorable therapeutic index compared to staurosporin,[2] which is another known PKC inhibitor, but still lacks specificity towards other protein kinases.

The literature contains several examples of total syntheses of balanol[3-5] in solution and some analogues with improved selectivity.[6-8] However, all syntheses reported numerous synthetic steps and a level of complication in the applied chemistry that is not yet compatible with solid-phase organic synthesis and combinatorial chemistry.

Therefore, we have approached the solid-phase syntheses of balanol analogues using a rather simplified synthetic scheme for our initial library construction. Thus, balanol was broken into the three most obvious key building blocks: a mono-protected diacid, a protected amino alcohol and a benzoic acid derivative (Scheme 1). The appropriately protected benzoic acid derivatives and amino alcohols are either commercially available or relatively easily synthesized whereas the supply of mono-protected diacids is rather limited. However, recently we have developed methods for the synthesis of such mono-protected diacids.[9]

In order to implement this synthesis on solid-phase, the outcome and optimization of the key coupling reactions were followed by *cleave-&-analyze* technique.[10] As depicted in Scheme 2, the first step could in principle be accomplished by the reaction of the hydroxymethyl polystyrene resin (Wang resin) with a symmetrical anhydride. However, only few symmetrical anhydrides are available and fewer would be relevant for the synthesis of balanol analogues. Likewise, we tested the reaction using different anhydrides, but only phthalic anhydride (**2**) gave good yield of support-bound phthalic acid (**3**). Therefore, we examined esterifications of the hydroxymethyl polystyrene resin with mono-allylated 2,6-naphthalene dicarboxylic acid (**4**) using several coupling methods and reaction conditions (Table 1). We found that esterifications using 1-(2-mesitylene-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) and N-methylimidazole (NMI) in dichloromethane (DCM) resulted in quantitative coupling as previously reported for anchoring amino acids to hydroxylated resin.[11] Deallylation of the support-bound mono-allylated carboxylic acid proceeded in high yield using conditions known from peptide synthesis.[12]

Table 1. Solid-Phase Esterification of Wang Resin (hydroxyl-functionalized resin).

Carboxylic anhydride or acid	Reaction conditions	% Yield
2	10 eq. **2**, 8,8 eq. Et_3N, 1,2 eq. DMAP	quant.
4	1,5 eq. **4**, 2 eq. DCC	13
4	1,5 eq. **4**, 1,1 eq. DCC, 2 eq. HOBT	17
4	2 eq. **4**, 5 eq. DCC, 0,5 eq. DMAP	25
4	1,5 eq. **4**, 1,1 eq. Ph_3P, 1,1 eq. DEAD	4
4	2 eq. **4**, 2 eq. MSNT, 8 eq. NMI	quant.

Next step in the solid-phase synthesis of the balanol analogues was the esterification of the support-bound free carboxylic group. We found the method using MSNT/NMI in DCM to be superior for esterification of carboxylated resin and couplings proceeded in very high yield for both primary and secondary alcohols and for both support-bound phthalic (**3**) and naphthalic acids (**5**, see Table 2).

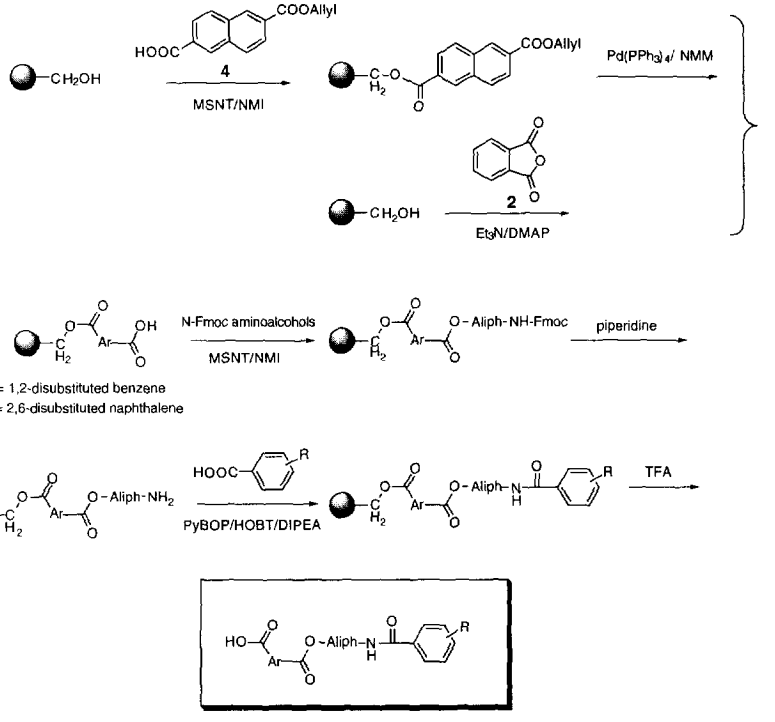
The removal of the Fmoc-function from the amino function was quantitative and the following benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP)/N-hydroxybenzotriazole (HOBT)/diisopropylethylamine (DIPEA)[13] acylation using the benzoic acid derivatives went quantitatively according to the 2,4,6-trinitrobenzenesulfonic acid (TNBS) analysis.[14]

For the initial synthesis of a library of balanol analogues, we selected two different mono-protected aromatic diacids, four different Fmoc-protected amino alcohols and four different benzoic acid derivatives. Using the *split-and-mix* synthesis,[15-16] we were able to synthesize four sub-libraries of each eight different balanol analogues in theoretically equimolar amounts as inherent of a true *split-and-mix* experiment.

The products were cleaved by TFA/DCM (1:1) and analyzed using diode-array LC-UV and LC-MS to identify the constituents of each of the four sub-libraries.[17]

Table 2. Solid-Phase Esterification of Support-Bound Carboxylic Acids [phthalic acid (**3**) and 2,6-naphthalene dicarboxylic acid (**5**)] with N-Fmoc Protected Amino Alcohols (abbreviated: A).

Support-bound carboxylic acid	N-Fmoc protected amino alcohol	Reaction Conditions	% Yield
3	2-Fmoc-amidoethan-1-ol	1,5 eq. A, 1,1 eq. Ph_3P, 1,1 eq. DEAD	0
3	2-Fmoc-amidoethan-1-ol	1,5 eq. A, 2 eq. DCC	31
3	2-Fmoc-amidoethan-1-ol	1,5 eq. A, 1,1 eq. DCC, 2 eq. HOBT	14
3	2-Fmoc-amidoethan-1-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	quant.
3	*trans*-4-Fmoc-amidocyclohexan-1-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	quant.
3	1-Fmoc-amidopropan-2-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	quant.
3	2-Fmoc-amidobutan-1-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	quant.
3	3-Fmoc-amidopropan-1-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	quant
5	2-Fmoc-amidoethan-1-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	quant.
5	*trans*-4-Fmoc-amidocyclohexan-1-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	74
5	1-Fmoc-amidopropan-2-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	91
5	2-Fmoc-amidobutan-1-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	86
5	3-Fmoc-amidopropan-1-ol	2 eq. A, 2 eq. MSNT, 8 eq. NMI	82



Synthetic strategy of balanol-analogues

Ar = 1,2-disubstituted benzene- or 2,6-disubstituted naphthalene-; Aliph = aliphatic backbone of amino alcohols

N-Fmoc amino alcohols = 1-Fmoc-amidopropan-2-ol, *trans*-4-Fmoc-amidocyclohexan-1-ol, 1-Fmoc-amidopropan-2-ol, 2-Fmoc-amidobutan-1-ol; Fmoc = 9H-fluorene-9-ylmethoxy-carbonyl ; R = -4-CH_2OR', -4-NO_2, -4-OCH_3, -3-F (R' = H or DMT).

Scheme 2

In conclusion, the rather complicated structure of the natural product balanol has been broken down to three relatively simple building blocks compatible with solid-phase synthesis using a retrosynthetic analysis. We have found that esterifications of both support-bound carboxylic acids or alcohols during solid-phase synthesis using MSNT/NMI mediated esterifications led to reactions with quantitative or nearly quantitative yields. In addition, we are currently expanding the number and chemical diversity of our building blocks to create larger libraries of balanol analogues. These libraries are expected to contain both genuine (-)-balanol and some closely related structural analogues together with a large numbers of very different structures as inherent of a combinatorial library.

Acknowledgments

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17. In the sub-libraries where R = -4-CH_2OH, -4-NO_2, -4-OCH_3, -3-F, respectively 6, 7, 7 and 7 balanol-structures were identified as distinct peaks in the LC-UV/MS analyses. The remaining five balanol analogues containing either one of the isomeric building blocks 1-amino-propan-2-ol or 3-amino-propan-1-ol were missing due to yet unclarified reasons (precipitation or co-elution in HPLC are likely possibilities). Further studies will be performed using non-isomeric structures to facilitate LC-MS identification, preferably in combination with a non-UV based HPLC detection system.

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