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A Novel Carboxy-functionalized Photocleavable Dinucleotide Analog for the Selection of RNA Catalysts

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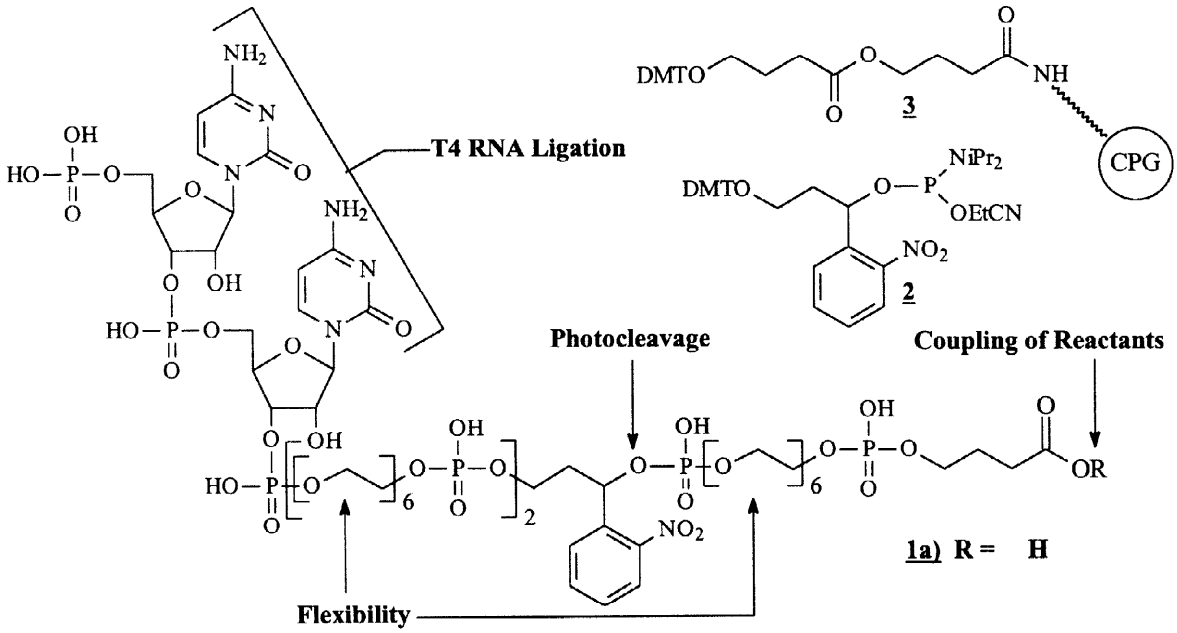
Abstract: A new multifunctional dinucleotide analog is synthesized for the application in *in vitro* selection experiments with linker-coupled reactants. For this purpose it contains a 5'-pCC ligation site, three flexible hexaethylene glycol spacers, a photocleavable *o*-nitrobenzyl unit and a 3'-terminal carboxy-function which can be derivatized with potential reactants as demonstrated with three model compounds. © 1998 Elsevier Science Ltd. All rights reserved.

A crucial step in the application of *in vitro* selection schemes[1] to the isolation of new catalysts from combinatorial RNA libraries is the attachment of small organic molecules to RNA transcripts via flexible linkers[2]. Recent success of selection experiments using such modified RNA libraries[3] has greatly increased the interest in RNA-linker conjugates containing a variety of organic molecules.

We have recently described a modified dinucleotide as a convenient tool for *in vitro* selection[4]. This analog could be derivatized with a broad range of activated N-hydroxysuccinimide esters and then enzymatically ligated to RNA transcripts. However, since the site-specific introduction and/or activation of a carboxyl group is sometimes troublesome (as in the case of nucleoside phosphates), we decided to revert the coupling chemistry and the functionalities involved to expand the range of addressable molecules. Since several selection experiments have been prone to side reactions[5] we also incorporated a photocleavage site within the linker in order to suppress the selection of these by-products[4]. This was realized by the synthesis of compound **1a** providing all the necessary features in one molecule:

- a 5'-phosphorylated dinucleotide for recognition by T4 RNA ligase,[6]
- three hexaethylene glycol units as flexible linkers ensuring optimal positioning of RNA and coupled reactant,[7]
- an *o*-nitrobenzyl moiety serving as photocleavage site,[8]
- a 3'-terminal carboxyl group for the coupling of amino-functionalized reactants.



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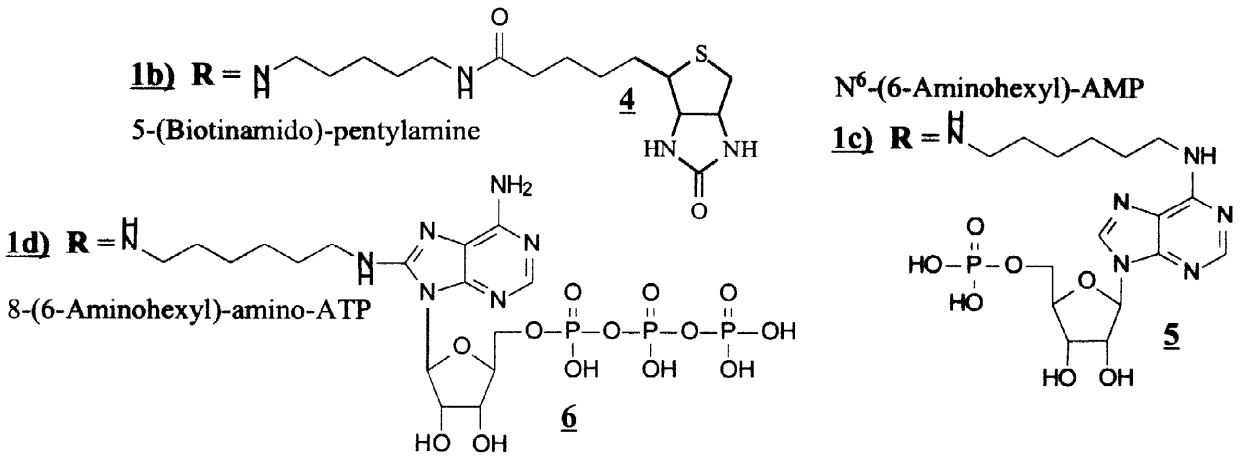

Compound **1a** was synthesized by automated phosphoramidite chemistry using solid support[9] **2** generating a 3'-carboxyl group and the photocleavable building block[8] **3** as well as standard phosphoramidites[10a]. Treatment with 33% aqueous triethylamine for 12h at 60°C prior to standard deprotection conditions (NH_3) avoided amide formation, and after desilylation **1a** was purified by HPLC in 15% total yield. Characterization by UV-MALDI-TOF-MS confirmed the correct total mass at 2084.6 g/mol (calc.: $[M-H^-]$ = 2084.1 g/mol) as well as the expected photocleavage products ([COOH-containing part $-H^-$] = 527.4g/mol; calc. = 527.1g/mol) thereby corroborating the photocleavability.

Purified **1a**[10b] was then activated with EDC and reacted with the corresponding amines of **4** and **5**. The resulting products were purified and analyzed by PAGE, HPLC and MALDI-MS[10c] showing the expected gel shifts, higher retention times and the calculated masses. Isolation after PAGE and HPLC yielded 30% and 10% of **1b** and **1c** whereas coupling of the ATP derivative **6** to compound **1a** proceeded with 25% conversion as determined by PAGE. The described dinucleotide analog therefore appears to be a superior alternative for the attachment of reactants so far out of reach.

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10. a) **1a** was synthesized on a Pharmacia Gene Assembler Plus in a 1.3µmol DMT-on scale using chemical phosphorylation and desilylation as in [4]. b) For sensitive electrophoretic analysis, **1a** was partially 5'-^{32}P labeled by dephosphorylation with shrimp alkaline phosphatase and rephosphorylation with γ-^{32}P-ATP and T4 polynucleotide kinase. c) 2µmol of the respective amine (**4**, **5**, or **6**), 20nmol of **1a**, and 1µmol of 0.25M EDC were reacted in 4µl 2M imidazole/HCl pH=6 for 30h, and with 2µl of fresh 0.25M EDC for another 4h. Reaction mixtures were applied to a 20% denaturing gel, the products were identified by autoradiography and eluted in water. After desalting on a 4ml sephadex G10-column, lyophilized products were purified on a HPLC C-18 column (Beckman) with 30% acetonitrile in 0.1M TEAAc pH=7. Masses obtained: **1b**: **4**-containing fragment = 837.5 g/mol (calc. = 837.6 g/mol); **1c**: **5**-containing fragment = 955.7 g/mol (calc. 955.5 g/mol).