Self-Assembly

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Cooperative Self-Assembly of Adenosine and Uridine Nucleotides on a 2D Synthetic Template**

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This paper describes a two-dimensional chemical system in which a divalent "template" guides and controls the stepwise and cooperative self-assembly mediated by base pairing of adenosine and uridine nucleotides.

Multiple hydrogen bonding and base pairing constitute one of the most widely studied classes of noncovalent interactions in supramolecular chemistry.[1] The precision with which nature utilizes complementary weak bonding to guide self-assembly of complex structures remains a fascinating challenge for surface chemistry that aims to develop novel approaches to interfacial sensing and nanofabrication.[2] To date, attempts to use base pairing as a basis for planar molecular-recognition systems have focused on self-assembled monolayers (SAMs)[3,4] or Langmuir monolayers[5] that are formed from compounds bearing nucleobases or their synthetic analogues. Although exceptionally useful in the precise vertical alignment of oligo/polynucleotides on solid supports,[6] the SAM-based methods are unsuitable for lateral tailoring of recognition surfaces with different types of bases. This is mostly because of steric hindrance and phase separation of monolayer constituents.[3,7] On the other hand, Langmuir monolayers enable fine-tuning of steric conditions in highly ordered films comprised of different entities at air/water interfaces,[8] but their practical applicability is severely limited. Herein, we use a novel surface-design scheme that combines the advantages of SAM-based and Langmuir-Blodgett (LB) monolayer approaches. This allows for precise harboring of arbitrary combinations of complementary nucleotides in planar films through sterically induced, coop-

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erative self-assembly on a surface presenting divalent bis-(Zn^{II}–cyclen) complexes.[9–12] These complexes have been previously used for selective recognition of thymidine and uridine bases in polynucleotides and natural DNA.[13] Furthermore, they have a use as highly efficient divalent receptors for thymidine and uridine nucleotides that interact with the bis-cyclic host through simultaneous binding of terminal phosphate and imide groups to complementary macrocyclic fragments.[14] In our system, the selective formation of bis(Zn^{II}–cyclen)-nucleotide aggregates promotes cooperative nucleotide self-assembly on a recognition surface.

We ordered amphiphilic bis(Zn^{II}–cyclen) derivatives (hereafter, Zn^{II}–BC) into a planar matrix through LB transfer of Zn^{II}–BC monolayers from an aqueous subphase onto a gold-coated surface covered with a loosely packed SAM of octanethiol (Figure 1). This simple combination of SAM and

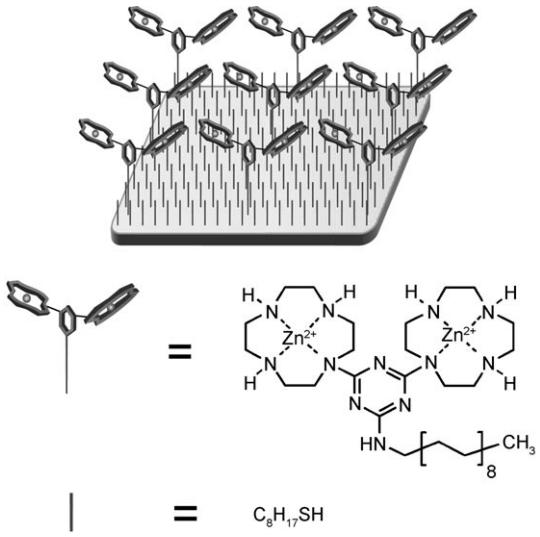

Figure 1. Schematically drawn structure of a SAM-supported monolayer of Zn^{II}–BC on a gold-coated surface.

LB techniques preserves a uniform order of the precursor monolayer and gives a stable, interdigitated bilayer with macrocyclic fragments exposed to the solution. According to our preliminary studies, Zn^{II}–BC immobilized in such a film is capable of binding nucleotide constituents modeled with uracil and an inorganic phosphate dianion while being inactive to adenine or monoanionic phosphates (for details see the Supporting Information).[15] The nucleotide assembly on functionalized SAM/LB plates was investigated in aqueous solutions of monovalent adenosine 5′-mono-, di- or triphosphates (hereafter, 5′-AXPs, where X = M (mono), D (di), or T (tri)) and/or divalent uridine 5′-mono-, di- or triphosphates (5′-UXPs, where X = M (mono), D (di), or T (tri)) at pH 7.5 through surface plasmon resonance (SPR) as a most appropriate tool for real-time monitoring of binding events.

The SPR sensograms in Figure 2 a,b illustrate typical kinetics of the stepwise assembly of 0.02 mM 5′-AXPs and 5′-UXPs for different orders of nucleotide addition (i.e., UTP probing followed by the subsequent addition of ATP and

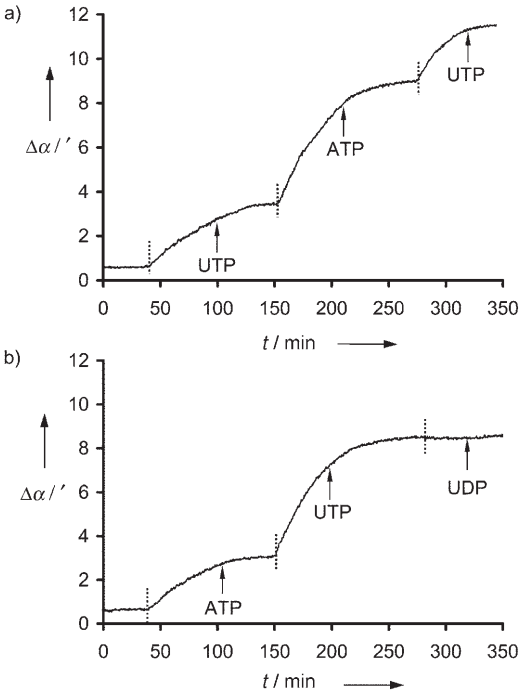

Figure 2. SPR sensograms for stepwise adsorption of a 5′-ATP/5′-UTP binary combination on a SAM-Zn^{II}–BC surface. a) The experiment started by probing with UTP (0.02 mM) followed by subsequent addition of ATP (0.02 mM) and of UTP (0.02 mM), b) the "vice versa" subsequent binding of ATP (0.02 mM) and UTP (0.02 mM) finalized by probing with UDP (0.05 mM); pH 7.5.

UTP (a) and adsorption of ATP, followed by UTP and UDP(b)).

Initially, Zn^{II}–BC surfaces responded slowly to both types of nucleotides. The first-stage binding translated into an SPR maximal response, $\Delta\alpha_1$, that falls within a range of 1.8–2.7 angle min;[16] the absolute values of $\Delta\alpha_1$ increased with the molecular weight of the measured nucleotides.[15] In addition, the kinetics and values of $\Delta\alpha_1$ determined for 5′-AXPs were similar to those measured for similar 5′- UXPs (here, 2.5–2.7 angle min for UTP and ATP).

After the completion of initial adsorption, similar probes of complementary nucleotides (0.02 mM) were added. The kinetics of secondary binding of either 5′-AXP or 5′-UXP at the surface with an already-adsorbed complementary partner was very different from that of initial recognition—the SPR signal increased rapidly and reached its maximal value $\Delta\alpha_2$, which was twice that of $\Delta\alpha_1$. Again, the $\Delta\alpha_2$ value varied from 3.6 to 5.4 angle min in proportion to the nucleotide's molecular weight, and the magnitudes of responses to 5′-AXP and 5′-UXP were similar (5.4 and 5.3 angle min for ATP and UTP, respectively). Importantly, because the magnitude of the SPR signal is proportional to the amount of adsorbed analyte, it appears that the initial binding of one molecule of either monovalent 5′-AXP or divalent 5′-UXP by Zn^{II}–BC film promotes further assembly of not one but two complementary molecules.

Furthermore, when equilibrated after secondary binding, the sensing surfaces were still capable of specific recognition; the films responded to the nucleotides that were comple-

mentary to the one bound at the second stage (Figure 2a). Moreover, maximal third-stage responses $\Delta\alpha_3$ were close to $\Delta\alpha_1$, that is, the efficiency of final adsorption was comparable with that observed at the initial stage. On the other hand, addition of a nucleotide containing a base similar to that used in second-stage binding caused no significant increase in the SPR signal even at increasing concentrations (Figure 2b). The specificity of the third-stage responses and their absolute values suggest that this stage proceeds with complementary recognition of incoming nucleotides (UTP on Figure 2a) by only half of the potentially available complementary partners already present on the surface (ATP on Figure 2a).

To understand the mechanism of nucleotide assembly, we make several observations related to the inhibition of secondary binding events. Firstly, as illustrated by Figure 3a,

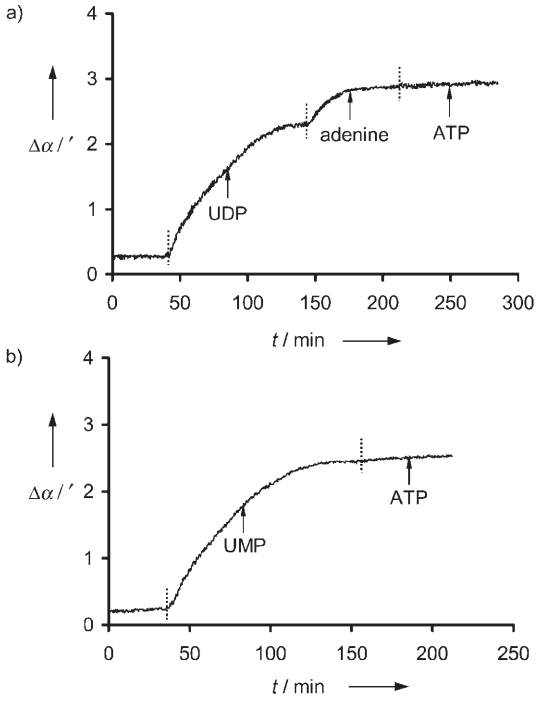

Figure 3. Inhibition of secondary recognition of 5′-ATP at SAM-Zn^{II}–BC sensing film. a) Initial adsorption of 5′-UDP (0.02 mM) at pH 7.5 followed by subsequent addition of adenine (0.05 mM; pH 7.9) and 5′-ATP (0.02 mM), b) the assembly of 5′-UMP (0.02 mM) followed by the probing with 5′-ATP (0.02 mM).

the protective binding of adenine to the Zn^{II}–BC monolayer with initially adsorbed 5′-UDP inhibited further assembly of 5′-ATP on the surface. Importantly, the response, $\Delta\alpha_2$, to adenine (which does not interact itself with Zn^{II}–BC) was almost exactly three-times lower than $\Delta\alpha_1$ for UDP. As adenine's molecular weight is also three-times lower than that of UDP, the registered signal difference corresponds to a 1:1 UDP–adenine binding ratio. The inhibitory effect of this binding on further 5′-AXP recognition suggests that phosphate coordination is the first-stage binding mode even for potentially divalent 5′-UXPs (except for UMP, which is discussed below); moreover, the immobilized nucleotides are well preorganized for the interactions with suitable complementary partners. The effect also points to the mediating role of base pairing in secondary recognition of complementary nucleotides—it is hindered when complementary H-bonding is "switched off".

The secondary recognition of adenosine phosphates was also inhibited by preliminary adsorption of UMP—the smallest divalent nucleotide studied herein (Figure 3b).[18] The distance between cyclic "heads" of Zn^{II}–BC is presumably well matched to UMP's molecular geometry thus allowing for simultaneous intramolecular coordination of phosphate and imide groups to the neighboring macrocycles. The surface with bound UMP becomes inactive with respect to other incoming nucleotides as both macrocyclic moieties in Zn^{II}–BC are occupied and therefore, the UMP nucleobase involved in this divalent binding mode is unavailable for base pairing.

These observations support a mechanism involving stepwise self-assembly of complementary nucleotides at Zn^{II}–BC monolayers (Figure 4). Specifically, irrespective of the nucleotide used, the first binding event is mediated by the coordination of the terminal phosphate to one of the "heads" of the Zn^{II}-BC template (Figure 4a). The geometry of the Zn^{II}–BC molecule prevents simultaneous divalent binding of 5′-UXPs with a distance between the base and terminal phosphate longer than that in 5′-UMP. These first-step interactions, however, cause steric crowding at the opposite Zn^{II}–cyclen, thus shielding the free macrocycle from similar nucleotides and hindering their binding to the same Zn^{II}–BC molecule.

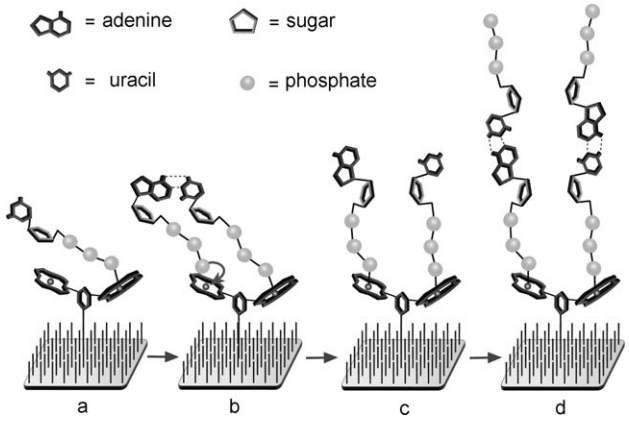

Figure 4. Schematic diagrams illustrating the possible mechanism of the stepwise self-assembly of complementary nucleotides on a SAM-Zn^{II}–BC template (one Zn^{II}–BC unit is shown). a) Phosphate attachment of a nucleotide to the one of Zn^{II}–cyclen units, b) base pairing accompanied by the guiding of terminal phosphate of complementary nucleotide to the opposite Zn^{II}–cyclen, c) firm attachment of the complementary nucleotide to Zn^{II}–BC and dissociation of the base pair, d) base pairing of incoming nucleotides with complementary partners already immobilized on the template.

Incoming complementary nucleotides recognize the already bound partner through base pairing.[19] Nucleotide coupling results in a spatial arrangement of the interacting components that favors simultaneous orientation ("guiding") of the terminal phosphate of a complementary nucleotide to

the free "head" of the Zn^{II}–BC molecule (Figure 4b). The cooperative action of the template and both nucleotides is, most likely, assisted by base stacking. Although the base stacking interactions stabilize the ordering layer significantly, the energetic contribution of hydrogen bonding is rather small compared with the phosphate-anion coordination. Therefore, dissociation of the base pair, exposing the hydrogen-bond donor and acceptor sites to the solution, is expected to be in equilibrium (Figure 4c). Disengaged bases, in turn, form complementary pairs with other incoming nucleotides.[20] This chain of consecutive interactions results in the formation of a bilayer structure that harbors equal amounts of complementary nucleotides (Figure 4d).

The above mechanism suggests that nucleotide self-assembly is independent from the formation path—that is, stepwise recognition of complementary nucleotides in an arbitrary order as well as their one-step self-assembly from an equimolar mixture should give the same nucleotide pattern on Zn^{II}–BC surfaces. To prove this assumption, we studied both continuous and interrupted adsorption of ATP/UTP equimolar mixtures (0.02 mM of each nucleotide) on Zn^{II}–BC templates. One-step ATP/UTP adsorption translated into a maximal increase in the SPR signal $\Delta\alpha_{\text{one step}} = 9.0$ angle min (data not shown; an SPR curve is given in the Supporting Information). This value is more than twofold greater when compared with $\Delta\alpha = 3.9$ angle min, which was measured for the adsorption of ATP from a solution with a double concentration of nucleotide (0.04 mM).[15] This correlates well to $\Delta\alpha_{\text{multistep}} = 10.4$, which was determined from Figure 2a for the stepwise binding of the ATP/UTP combination.

Figure 5 describes interrupted adsorption of a similar mixture, followed by stepwise surface probing with ATP (0.02 mM) followed by UTP (0.02 mM) solutions. The ATP/UTP mixed solution was replaced by one of ATP (0.02 mM) when $\Delta\alpha$ reached 6.5 angle min greater than $^1/_2\Delta\alpha_{\text{multistep}} \cong 5.2$ angle min. This value assumes that more than half of the nucleotides that constitute the final bilayer were already immobilized on a surface. After the completion of ATP binding, UTP (0.02 mM) was added. We observed almost equal SPR maximal signals for individual solutions; an overall increase in the SPR signal $\Delta\alpha_{\max}$ amounted to 9.4 angle min.

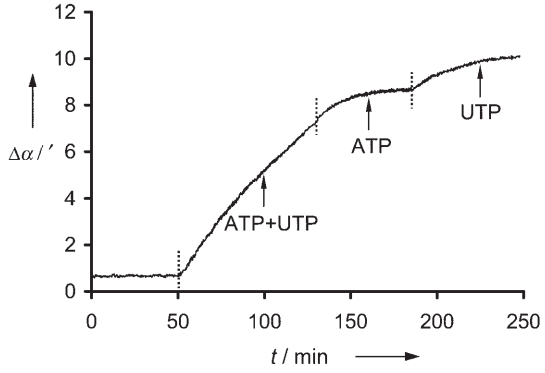

Figure 5. SPR sensogram for the interrupted adsorption of 5′-ATP/5′-UTP equimolar mixture (0.02 mM of each nucleotide) replaced by ATP (0.02 mM) at $\Delta\alpha = 6.5$ angle min and completed with the addition of UTP (0.02 mM).

Close agreement between maximal SPR responses registered for one-step and stepwise modes of assembly indicates that constant amounts of nucleotide bind to the surface irrespective of the procedure used. Some of the differences between $\Delta\alpha_{\text{one step}}$ and $\Delta\alpha_{\text{multistep}}$ may be attributed to steric crowding at the interface in more concentrated mixtures. Such crowding results in the formation of structures that are not-so-rigorously defined, presumably because some base pairs between the nucleotide layer attached to the template and the complementary top layer are uncompleted. Furthermore, equal SPR signals measured in subsequent probing with individual nucleotides in Figure 5 prove the alteration of the binding mode in the course of self-assembly; when the Zn^{II}–BC template is saturated with bound nucleotides and a bottom layer is formed, further completion of the bilayer structure proceeds through 1:1 base recognition.

In summary, we described sterically induced cooperative assembly of complementary nucleotides progressing through the chain of consecutive mono- and divalent interactions on a surface presenting artificial Zn^{II}–BC receptors. We believe that the approach utilized herein might be extended to other types of planar patterns of complementary nucleotides, as well as for the preparation of other artificial systems preorganized for efficient molecular recognition and further bottom-up self-assembly of synthetic molecules containing suitable fragments.

Experimental Section

All nucleotides (adenosine 5′-monophosphate, adenosine 5′-diphosphate disodium salt hydrate, adenosine 5′-triphosphate disodium salt hydrate, uridine 5′-monophosphate disodium salt hydrate, uridine 5′-diphosphate disodium salt hydrate, and uridine 5′-triphosphate trisodium salt hydrate) are of analytical-reagent grade and were obtained from Acros Organics (Belgium). Nucleotides and organic bases (uracil and adenine) were dissolved in water that was deionized to 16 MΩ cm resistivity and preliminarily adjusted to pH 7.5 by the addition of a small amount of sodium hydroxide; these solutions were used for SPR measurements immediately after their preparation.

Platform preparation: TF-1 glass supports covered with a Cr adhesion sublayer (5 nm) and a polycrystalline Au layer (50 nm) supplied by Analytical-μSystem were modified by immersion into an absolute ethanol solution of octanethiol (1 mM) for 2 min. The monolayers of Zn^{II}–BC were spread from 10^{-5} M chlorophorm solution onto basic subphase with pH 8.52, compressed to 17 mN m^{-1}, and vertically transferred (the down-stroke mode of transfer was applied) onto thiolated support at a constant speed of 0.5 mm min^{-1}.

SPR monitoring: The SPR Kretschmann-type spectrometer Biosuplar-2 (Analytical-μSystem; a light-emitting diode light source $\lambda = 670$ nm) equipped with a peristaltic pump (flow rate 0.3 mL min^{-1}) was used for kinetic monitoring; freshly prepared solutions were added in 15 mL portions to the pump vessel when the previous portion was nearly all used. The SPR cell was rinsed with pure water for 15–20 min prior to the addition of each next probe.

Other experimental details are given in the Supporting Information.

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[14] S. Aoki, E. Kimura, *J. Am. Chem. Soc.* **2000**, *122*, 4542. The authors mentioned an effect of geometrical mismatch on ditopic binding of various U(T)XP's at *meta*- and *para*-xylyl-bis(Zn^{II}–cyclen); *para* isomers with a longer distance between cyclic fragments acted as more efficient divalent receptors than *meta* dimers for the most of investigated nucleotides. However, the *meta* isomer was found to be a more appropriate host for 2′-UMP as the intercycle distance in the *para* isomer was too long for simultaneous coordination of both phosphate and imide groups of the nucleotide.

[15] See the Supporting Information for details of the synthetic procedure for the amphiphilic bis(Zn^{II}–cyclen) derivative, the data for IR, UV/Vis, ^{1}H NMR spectrometry, ^{13}C NMR spectrometry, and MS for the final and intermediate products, the formation procedure for Langmuir monolayers and the LB monolayer of Zn^{II}–BC, and surface pressure–area isotherms. Additionally, cyclic voltammagram (CVA) data on SAM and SAM/Zn^{II}–BC films with an estimated surface coverage and SPR data on the adsorption of other nucleotide combinations that are not described in the main text are also included in the Supporting Information.

[16] Immediate shifts (ones faster than SPR scan) in SPR signals are attributed to the difference in refractive indexes of the analyzed solutions as well as those portions of kinetics curves, which correspond to the rinsing of the SPR cell with water at the end of each stage, were cut from the graphs for clarity. However, the $\Delta\alpha_i$ values that are given account for the "rinsing" decrease in the SPR signal that normally fell within a range of 0.1–0.3 angle min; maximal "rinsing" decrease was observed after first-stage adsorption.

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[18] The two-stage binding of these nucleotides in reversed order (UMP after ATP) followed the recognition pattern similar to that observed for all other binary combinations—slow initial adsorption of ATP promoted faster and more pronounced responses to UMP (the SPR curve is given in the Supporting Information).

[19] The enthalpic contribution of hydrogen bonds in a single base pair in aqueous solution is too small for stable aggregate formation. However, polarities at interfaces are different from bulk solution. Furthermore, owing to the dramatic change in polarity from the aqueous to the lipid phase, the dielectric constant in the critical recognition zone close to the lipid surface is dramatically lowered with the consequence of drastically enforced electrostatic and hydrogen bond interactions (by a factor of $\approx 10^6$). For an overview and examples, see Ref. [8]; for recent examples applying this effect, see: a) Peptide and protein recognition at mono- and bilayers. Equimolar mixed monolayers of a diglycine and a guanidinium amphiphile were shown to bind free dipeptides by a guanidinium carboxylate interaction and stable antiparallel hydrogen bonds among the peptide chains. K. Ariga, A. Kamino, X. Cha, T. Kunitake, *Langmuir* **1999**, *15*, 3875 – 3885; b) Size matching of amino acid residues in host and guest leads to a certain sequence selectivity for aliphatic peptides. Synthetic bilayers were evenly covered with phosphate anions to bind basic proteins in water at millimolar concentrations. N. Kimzuka, A. Baba, T. Kunitake, *J. Am. Chem. Soc.* **2001**, *123*, 1764 – 1765; c) Matile reported a possible explanation for the cell-permeating activity of arginine-rich protein transduction domains, which have received considerable attention because they transport anionic substrates across membranes. Extensive phase-transfer experiments with liquid and bilayer membranes support their concept of "counteranion scavenging". N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2003**, *125*, 14348 – 14356; d) Koh immobilized BSA on gold surfaces by non-covalent interaction with calix[4]arene derivatives carrying carboxylate groups at their upper rims. The new SAMs were examined with SPR and showed a higher BSA concentration on those surfaces covered with carboxylates rather than those with ester groups. M. Lee, W. G. An, J.-H. Kim, H.-J. Choi, S.-H. Kim, M.-H. Han, K. Koh, *Mater. Sci. Eng. C* **2004**, *24*, 123 – 126; e) Membrane-embedded synthetic receptors showed nanomolar affinities to proteins. R. Zadmard, T. Schrader, *J. Am. Chem. Soc.* **2005**, *127*, 904 – 915; S. Kolusheva, O. Molt, M. Herm, T. Schrader, R. Jelinek, *J. Am. Chem. Soc.* **2005**, *127*, 10000 – 10001.

[20] A major energetic contribution for assembly comes from base-stacking, whereas hydrogen bonding contributes to the selectivity of the process.

