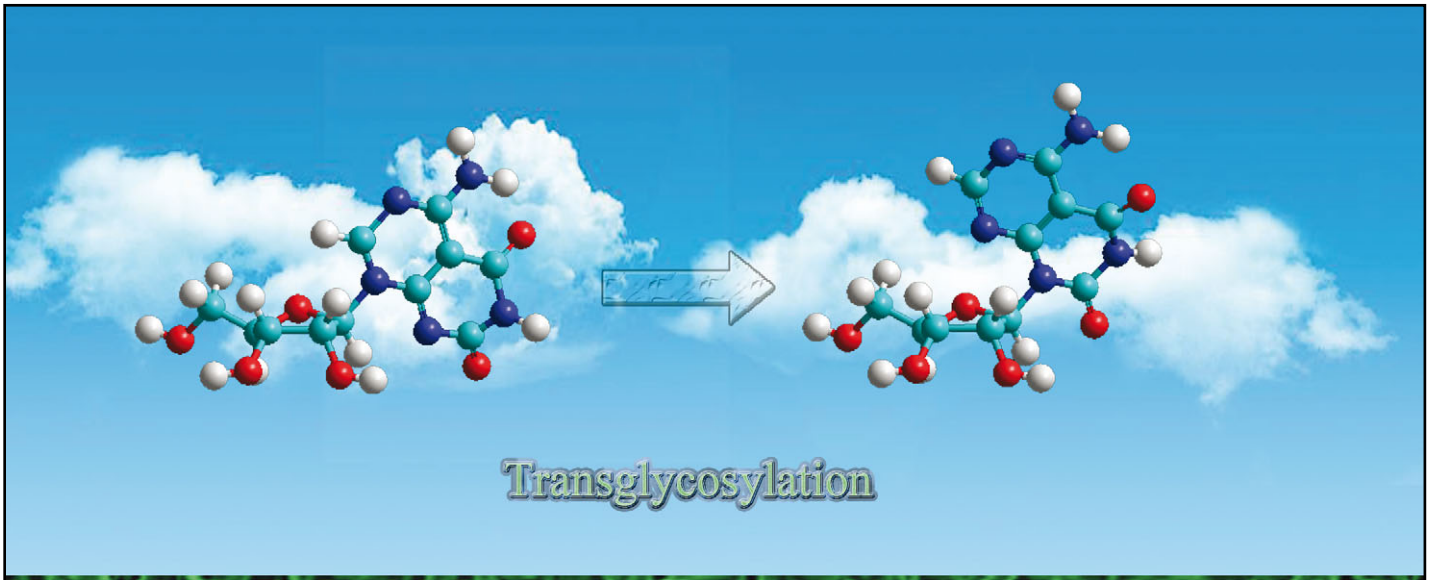

Joint research work from the Laboratory of Ethnopharmacology, West China Hospital and the State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University

Micro-flowers changing to nano-bundle aggregates by translocation of the sugar moiety in Janus TA nucleosides

The Janus-type TA nucleosides were designed and synthesized. The translocation of the ribose from N8 to N1 leads to the formation of entirely different superstructural morphologies, micro-flowers for J-AT and nano-bundles for J-TA.

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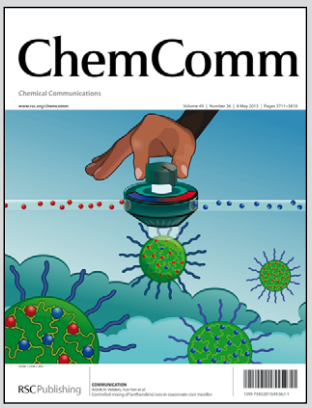

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Micro-flowers changing to nano-bundle aggregates by translocation of the sugar moiety in Janus TA nucleosides†

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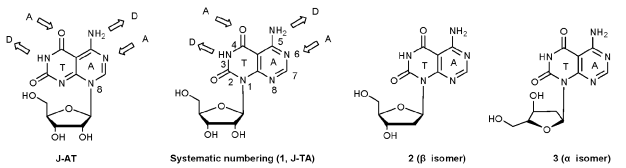
We designed and synthesized the Janus-type TA nucleosides (1–3) by using a transglycosylation protocol. Surprisingly, the subtle translocation of the ribose from N8 to N1 by about 230 pm in space leads to the formation of entirely different shaped superstructures, micro-flowers for J-AT and nano-bundles for J-TA.

Since the establishment of supramolecular chemistry,[1] non-covalent interactions have been extensively employed to construct complex architectures in nanotechnology.[2] One of the major challenges in this field is to create predictable and controllable structures by molecular self-assembly.[3] To accomplish this, inorganic or organic molecules, synthetic polymers and biopolymers (peptides, DNA, RNA) have been investigated over the past decades.[4] Due to their unique complementarities, equipped with solid-phase synthetic methods and an enzymatic toolkit, oligonucleotides (DNA or RNA) have become one of the most versatile candidates for creating various programmable and manageable superstructures.[4fg,5] As the building blocks of DNA/RNA, nucleosides have also been exploited for this purpose.[6] Besides canonical purines and pyrimidines, other heterocycles have also been developed to form supramolecular structures which have been widely applied in the field of nanomaterials.[7,8]

To expand further, the Janus-type tridentate GC (J-GC) and bidentate AT and HC nucleosides were designed and synthesized by our group.[9] We found that the J-GC ribonucleoside can form nano-bundles and the Janus-type AT ribonucleoside (J-AT) can form flower-like superstructures in DMF solution.[9c] However, the J-AT ribonucleoside is not a perfect mimic of adenosine or thymidine because its ribose is connected to the N8 atom which is on the adenine ring, different from the natural nucleosides. Hence, after many tries we finally synthesized, through transglycosylation reaction, the desired bidentate Janus-type TA nucleosides (J-TA, **1–3**) which have the same geometrical arrangements as the natural ones with the sugar residues attached to the N1 atom on the thymine ring (Scheme 1). Although compounds J-AT and J-TA (**1**) have the identical two-faced H-bond arrays and the same sugar counterparts, the subtle translocation of the ribose from N8 to N1 by about 230 pm in space leads to the formation of entirely different shaped superstructures, micro-flowers for J-AT and nano-bundles for J-TA (**1–3**), which we would like to report herein.



Scheme 1 The structures of bidentate J-AT and J-TA nucleosides. The arrows of D represent the hydrogen donors; the arrows of A represent the hydrogen acceptors.

For the synthesis of compound **1**, initially, the Vorbrueggen glycosylation reaction was adopted.[9d,10] But, various conditions all led to the formation of compound **6** instead of compound **8** (Scheme S1, ESI†). The reason for the glycosylation reaction regioselectivity is that N8 of key intermediate **5** is more active than N1, as illustrated by its larger *f* value of Fukui functions (Fig. S1, ESI†), which are descriptors of chemical reactivity computed using density functional theory (DFT). To overcome this problem, we also tried a different route: construction of the second pyrimidine ring from monocyclic pyrimidine nucleoside **10** (Scheme S2, ESI†). Unfortunately, the target compound **8** was not formed probably because the sugar exerts certain steric hindrance in the ring closure process.

The transglycosylation reaction[11] has not yet been utilized in the field of pyrimido[4,5-*d*]pyrimidine nucleoside synthesis. Here, we investigated the feasibility of this translocation reaction to synthesize compound **1** (Scheme 2). The benzoylated **6**[9d] was treated with HMDS and xylene to afford the silylated intermediate **7**. Next, compound **7** was rearranged to afford compound **8**. For this step,

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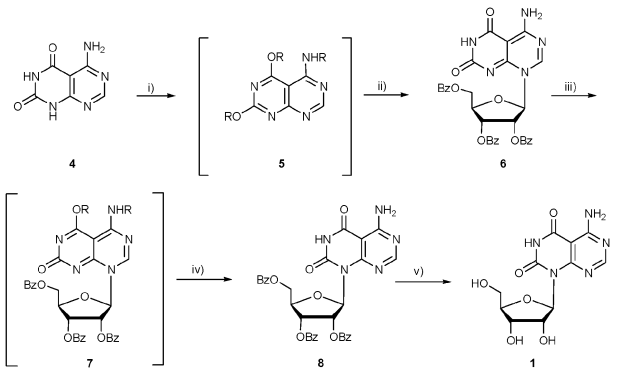
† Electronic supplementary information (ESI) available: Full experimental details for all compounds, VT-NMR, molecular modelling and microscopy preparations and experiments. See DOI: 10.1039/c3cc41383g

‡ These authors contributed equally to this study.



Scheme 2 Synthesis of the J-TA nucleoside (**1**): R = TMS; (i) and (ii) steps;[9d] (iii) HMDS, xylene, reflux; (iv) dry acetonitrile, TMSOTf, 75 °C, 2 h, 51%; (v) 0.5 M NaOMe, reflux, 82%.

diverse Friedel–Crafts catalysts ($SnCl_4$, TMSOTf), reaction solvents ($ClCH_2CH_2Cl$, CH_3CN, and mixture solvents), reaction times (1 h, 2 h, 6 h, 1 d and 3 d) and reaction temperatures (25 °C, 50 °C, 75 °C and 100 °C) have been tested and finally the conditions of dry acetonitrile, TMSOTf, 75 °C, 2 h were proved to be the best choice. Subsequently, the target compound **1** was obtained as a white powder by removing the benzoyl groups from compound **8**.

For the synthesis of 2′-deoxyribonucleosides (**2** and **3**), the same transglycosylation reaction was adopted (Scheme S3, ESI†). However, the anomeric mixture of **13a** (β) and **13b** (α) was difficult to separate by FC or recrystallization. After the toluoyl groups were removed it was also impossible to separate the α and β isomers. In order to get the pure anomeric 2′-deoxynucleosides, the 4,4-dimethoxytrityl (DMT) group was employed. When the DMT group was introduced into the 5′-OH group, compounds **14a** and **14b** could be separated by FC successfully. Afterwards, the DMT groups of **14a** and **14b** were removed and the target compounds **2** (β) and **3** (α) were obtained. The Nuclear Overhauser Effect (NOE) gave unambiguous evidence concerning the configuration of the anomeric center: 1′-H of **2** produced a strong NOE at 2′-H_a and 2′-H_b while 1′-H of **3** showed an NOE at 2′-H_a, and 3′-H simultaneously (Fig. S2 and S3, ESI†).

The structures of compounds **1–3** were characterized using NMR and HRMS. The coupling interaction between the C7 atom of the AT base and the 1′-H atom of the sugar provided the evidence that the sugar was attached to the N1 atom of the thymine ring (Fig. S4–S9, ESI†). The UV spectroscopy of compounds **1–3** showed a blue shift of 23 nm compared with J-AT, which was an additional indication of their different isomeric form (Fig. S10, ESI†). As reported for Janus-type derivatives before,[7h,9d] the variable temperature ^{1}HNMR (VT NMR) showed that the intermolecular and intramolecular hydrogen bonds exist for the self-assembly of these new J-TA nucleoside systems in DMSO (Fig. S11–S13, ESI†).

There are two possible ways to maintain the bidentate hydrogen bonding arrays for the normal adenosine and thymine base pair: the Watson–Crick base pair or the reverse Watson–Crick base pair; they will lead to the formation of either antiparallel-stranded or parallel-stranded DNA/RNA.[12] These two base pair motifs have little energy difference according to theoretical calculation (~1.3 kJ mol^{-1}).[13] For currently studied pyrimido[4,5-*d*]pyrimidine, DFT calculations predicted a rather small energy difference between these two motifs (~0.85 kJ mol^{-1}, Fig. S14, ESI†). If the Watson–Crick base pair is adopted a rosette structure will be formed; on the other hand if the reverse Watson–Crick base pair is adopted a linear tape structure will be formed (Scheme S4, ESI†). To see which one is dominant for compounds **1–3** in water, SEM, TM-AFM and TEM were carried out.

Compounds **1–3** and J-AT were dissolved in water (0.2 mg mL^{-1}), heated and cooled at room temperature for 48 h. Characterization of J-AT nucleosides using SEM showed that it can form micro-flower superstructures in water; the thickness of the flower leaves was about 120 nm (Fig. 1A and Fig. S15, ESI†), which is similar to the previous observation in DMF.[9c] There is no finer structure for this micro-flower observed under TEM. Considering their same base pair motifs, it is very interesting to investigate whether or not nucleosides (**1–3**) will form superstructures similar to Janus-type TA in water. As a matter of fact, completely different superstructures were found for them. When observed using SEM, these compounds formed nano-bundles; the length of the nano-bundles is about 20 μm; these nanobundles interweave to form three-dimensional networks whose overall shape is quite similar to that of collagens (Fig. 1B and Fig. S16–S18, ESI†).[14] When these samples were observed under TM-AFM, the same nanobundles were detected as well (Fig. S19–S21, ESI†). Then, the finer structure of these nano-bundles was revealed by TEM which indicated that these nanobundles consisted of nanotubes with an outer diameter of ~4 nm (Fig. 1C and Fig. S22–S24, ESI†). As reported for GC derivatives,[15] these nanotubes self-assemble through hierarchical non-covalent interactions. Firstly, a six-membered supermacrocycle was constructed by H-bonding through Watson–Crick base pairs. Indeed, DFT calculations revealed that the pattern of hexameric macrocyclic form of J-TA nucleosides is about 43.05 kJ mol^{-1} more stable than the corresponding linear arrangement formed by reverse Watson–Crick base

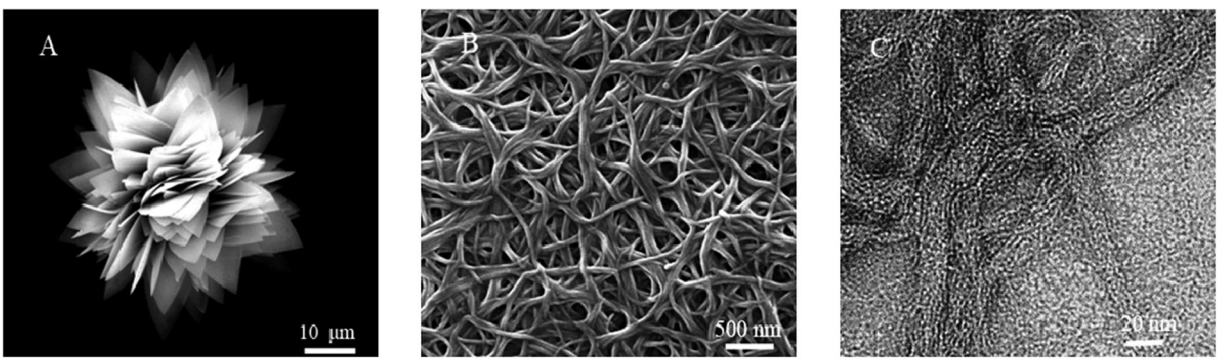


Fig. 1 Imaging of micro-flowers and nano-bundles by (A) SEM micrograph of J-AT; (B) SEM image of sample **1**; (C) TEM image of compound **1**.

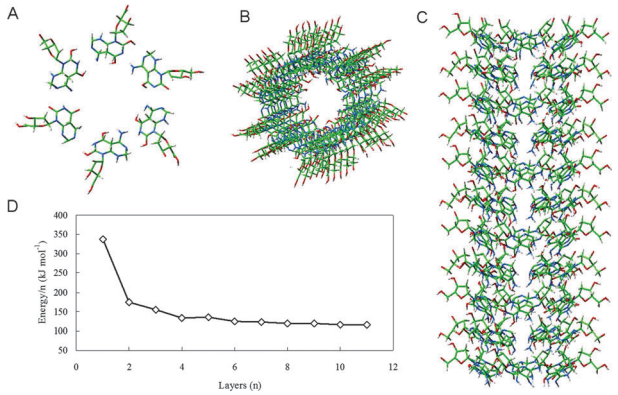


Fig. 2 Hierarchical self-assembly of compound **1**. (A) Six-membered super-macrocycle assembly of Janus molecules was constructed by H-bonds; (B) nanotubes were formed by stacking between layers (top views); (C) side views of the nanotubes (n = 12); (D) variation of energy per layer (E/n) with layer (n) stacking.

pairs (Fig. 2A and Fig. S25, ESI†). Secondly, these six-membered rings stacked layer by layer to form rosette nanotubes (Fig. 2B and C). The calculations at the ff03 force-field level were performed for the stacking up to n = 12 layers, showing that the stacking leads to extra stability for the rosette nanotubes (Fig. 2D and Fig. S26, ESI†) and the elongation of these nanotubes is energetically favourable. Previously, the GC base derivatives were reported by Fenniri and coworkers to form ~10% nanobundles by nanotubes. Here we have found that nearly 100% of nanotubes associate to form higher ordered nano-bundles. The difference is obviously due to the presence of sugars attached to the base, which will introduce additional hydrogen bonds between adjacent nanotubes. The hollow structure of nanotubes together with the densely packed nano-bundle networks formed by nucleosides **1–3** may have potential to be used for drug delivery systems and biomaterial engineering.

In summary, we have developed a viable route to synthesize the new pyrimido[4,5-*d*]pyrimidine Janus-type TA ribonucleoside (**1–3**) by transglycosylation reactions. Albeit J-AT and J-TA (**1**) have the identical two-faced H-bond arrays and the same sugar counterparts, the subtle translocation of the ribose from N8 to N1 (by about 230 pm in space) leads to the formation of drastically different shaped superstructures, micro-flowers for J-AT and nano-bundles for J-TA (**1–3**). Previously, we have shown that the modifications of base moieties can change the superstructures of these Janus-type nucleosides.[9c] The current phenomenon indicates that not only the base pair but the spatial arrangement of the sugar is also a deterministic factor to direct the three dimensional superstructure shapes. Based on these findings, we believe that, by further fine-tuning the structural parameters of these Janus-type nucleosides such as the functional groups, the torsion angle of the glycosyl bond (*anti*- or *syn*-conformation), the sugar puckering mode (North- or South-conformation), *etc.*, more beauties and complexities of the macro-world can be reproduced and find certain applications in the micro- or nano-world. These experimental and theoretical studies are currently under intensive investigations and will be published in the near future.

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