ChemComm

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Journal:	*ChemComm*
Manuscript ID:	CC-COM-05-2015-004212.R1
Article Type:	Communication
Date Submitted by the Author:	22-Jun-2015

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Journal Name

COMMUNICATION

5'-*Vs* 3'-end sugar conformational control in shaping up dinucleotides

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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The 5'-end N-sugar puckering is currently believed to govern intramolecular dinucleotide stacking. We demonstrate that, if this 5'-conformation is indeed important in shaping up dinucleotide structure, the 3'-end sugar conformation can either potentiate or cancel the stacking capacity induced by the 5'-end N-sugar conformation.

Nucleobase stacking, that when reduced to its simplest expression is defined as the mutual spatial arrangement and energetic of two interacting nucleobases, results from direct and induced electrostatic interactions.[1] Within nucleic acids, and in the absence of any environment interaction, nucleobase stacking is further influenced by complex, intimate, and divergent or convergent interactions imposed by the sugar-phosphate backbone.[1c,2] Stacking contributes to stability, folding and local conformational variability of nucleic acids. As such, it represents one of the keystones governing the biological properties of nucleic acids.[1c,3] Consequently, significant experimental and theoretical efforts have been achieved to understand the respective influence of every parameter that influences base stacking within nucleic acids with the underlying idea of ultimately designing modified sugar-phosphate backbone units to finely control nucleic acid structural/functional properties.[4] This approach could open new opportunities in the field of small functional nucleic acids.[5] Regretfully, nucleobase/sugar phosphate interactions are still far from being entirely known.

At the single-stranded dinucleoside monophosphate level, numerous studies have highlighted the impact of the 5'-end sugar puckering on the intramolecular nucleobase stacking[6] leading to the commonly accepted idea that the 5'-sugar conformation governs the dinucleotide stacking propensity.[7] Contrastingly, the 3'-end sugar conformation is generally believed to present a minor influence, if any. For a peculiar pairwise combination of nucleobases, ribodinucleotides generally present a higher stacked population than that of the corresponding deoxyribodinucleotides.[7c,8] In ribo- and deoxyribo-dinucleotides, the sugar conformation is usually predominantly North (N) and South (S), respectively.[7c,8a-c] Therefore, a N-N sugar conformational sequence is often considered to be a favorable parameter for an efficient base stacking in dinucleotides, a S-S sequence being less favorable. Stacking efficiency of mixed conformers has only been seldomly examined.

The dithymine deoxyribodinucleotide (thymidylyl(3',5')thymidine, TpT, **1**, Fig. 1) sugar conformation is only 26% N and 34% N at its 5'- and 3'-end, respectively.[9] TpT is known to present a limited base-stacked population[8c,8e,10,11] whose degree would be 12%[12] or 23%,[8f] depending on the studies. However, TpT analogues in which the N population at each extremity has simultaneously been gradually increased present a substantial intramolecular stacking enhancement.[13] Ultimately, the dinucleotide containing two N-locked sugars ($T_{LN}pT_{LN}$, **2**, Fig. 1) was estimated from CD differential spectra superimposition to be *ca* 3 to 4 times more stacked than **1** (Table 1).[14] Therefore, the stacking ability of **1** appears to be very sensitive to minute conformational changes of the sugar-phosphate backbone, and **1** can legitimately be proposed as a useful tool to approach the fine tuning of the influence of any conformational modification on the global dinucleotide stacking.

In the course of physicochemical studies currently performed in our Laboratory, we previously synthesized compound **3** ($T_{LN}pT_{LS}$, Fig. 1),[15] a dinucleotide N- and quasi S-locked (90% S)[16] at its 5'- and 3'-end, respectively. Having **3** in hands led us to question about its stacking capacity, with the expectation that its putative stacked population level would be similar to that of **2** since both compounds include a 5' N-locked

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† Electronic Supplementary Information (ESI) available: Experimental procedures, analytical data, NMR and CD melting spectra, SVD analysis. See DOI: 10.1039/x0xx00000x

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sugar. Surprisingly, we found the stacking population degree of **3** to be only 21% (see below), a value not significantly different from that of **1**. If **3** represents the first experimental evidence that an almost exclusive N-S form is able to stack, such low extend of stacking was highly deceptive. To understand this unexpected result, we decided to synthesize $T_{LN}pT$ and TpT_{LN} (**4** and **5**, respectively, Fig. 1), two analogues of TpT whose respective 5'- and 3'-end sugar conformation is locked in the N domain. The intramolecular stacking equilibrium of **3**-**5** was studied by electronic circular dichroism and analyzed with respect to the one of **1** and **2**.[14]

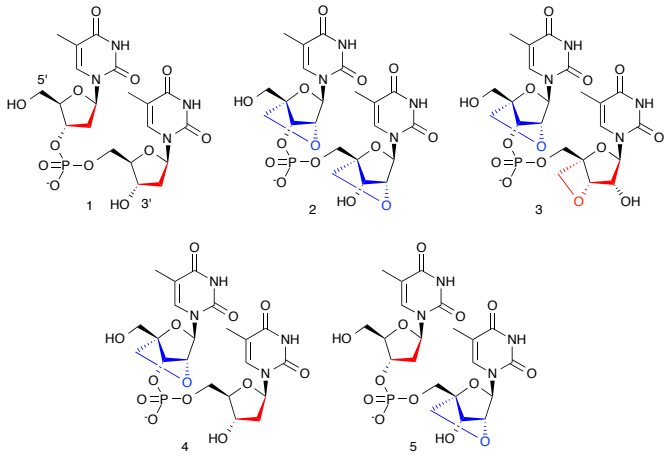

Fig. 1 Structure of TpT (**1**), $T_{LN}pT_{LN}$ (**2**), $T_{LN}pT_{LS}$ (**3**), $T_{LN}pT$ (**4**) and TpT_{LN} (**5**) (S-Sugar preference is indicated in red and N-locked sugar is indicated in blue).

Synthesis of **4** and **5** was performed from commercially available nucleosides using the phosphoramidite chemistry (see ESI†). The N conformation population of the deoxyribose residue of **4** and **5** was estimated to be 40 and 38% N, respectively.[17] Variable temperature CD spectra of **4** and **5** were recorded to evaluate their stacking level. The temperature-dependence of the CD spectra of **4** and **5** signed stacking interactions witnessed by the relatively high amplitude at long wavelength and large variation of the melting curves (Fig. S25 and S26, ESI†).

In solution, the stacking equilibrium of **1** exhibits a two-state model whose stacked population adopts a face-to-back[18] right-hand conformation[8e,8h,19] with a twist angle near 40°.[8f,12,20] and is mostly composed of S-S conformers in a rapid equilibrium with S-N conformers.[20] The presence of isobestic points on the melting CD spectra (Fig. S24, S25 and S26, ESI†) of **3**-**5** attested, as already observed for **1**[8h] and **2**,[14] a two-state model for their stacking equilibrium. In addition, the similarity of the shape of the adjusted CD differential (CD_{Diff}) spectra of **1**-**5** (Fig. 2) suggested that **3**-**5** present an overall face-to-back right-hand stacked geometry similar to the one of **1** and **2**. From a simple experimental adjustment calculation, the stacked population of **4** and **5** was estimated to be *ca* 2 to 3 and 1 to 2 times higher than that of **1**, respectively, whereas **1** and **3** displayed similar extend of stacking (Fig. 2, Table 1). CD melting data of **1**, **3**-**5**, and those previously reported for **2**,[14] were further analyzed by mathematical treatment. Singular value decomposition (SVD) analysis confirmed that two independent spectroscopic species were involved in their thermodynamic equilibrium (see ESI†).[21]

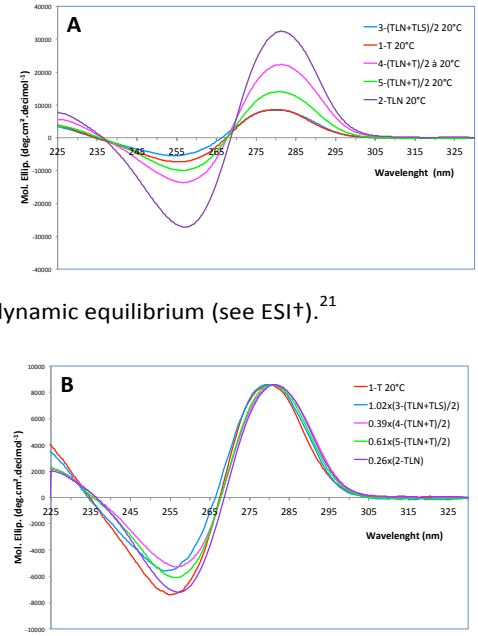

Fig. 2 CD differential (CD_{Diff}) spectra of **1**-**5** at 20°C (A), adjusted to **1** (B).

SVD-derived first temperature eigenvector fitting analysis[21b] and Taylor series curve fitting method[21b,c] were used to quantify the population of stacked species (see ESI†). Both methods afforded similar results and **1**-**5** were calculated to be 24, 71, 21, 43 and 26% stacked, respectively, at 25°C (Table 1).

Table 1 Estimated and calculated intramolecular stacking level in **1**-**5**

	1	**2**	**3**	**4**	**5**
5'-3' N-Sugar population (%)	26-34	100-100	100-10	100-40	38-100
Estimated stacking[a]	-	3 to 4[14]	*ca* 1	2 to 3	1 to 2
Calculated stacking[b]	24/25	70/71	22/20	43/43	26/26

[a] multiplication factor with respect to **1**
[b] in % at 298 K from SVD/Taylor analysis

These calculated values are consistent with those reported by Jafilan *et al.*[8f] for TpT and with our experimental CD adjustment for **2**-**5**.

Unsurprisingly, dinucleotides **4** and **5** exhibited a different stacked population level. The degree of stacked population of TpT_{LN} (**5**), whose 3'-sugar pucker is N-locked but whose 5'-pucker is predominantly S, was similar to that of **1** (whose unconstrained sugars are predominantly S) and **3** (whose

constrained sugars are 5'-N and 3'-S). In contrast, the amount of stacked population of T_{LN}pT (**4**), whose 5'-end sugar pucker is N-locked, but whose 3'-end pucker is predominantly S, was almost twice higher than that of **1**, but lower than that of **2**. These results confirm that an N conformation at the 5'-end of a dinucleotide can produce an enhancement of the stacking level. Indeed, stacking is moderately to dramatically increased when the 3'-end sugar is unconstrained and predominantly S, as in **4,** or N-locked, as in **2**. However, a 5'-N sugar pucker is necessary but not sufficient to promote dinucleotide stacking, which clearly crucially depends on the conformation of the 3'-sugar residue: if the 3'-sugar is quasi S-locked as in **3**, the 5'-end N-conformation is inefficient to induce any strong stacking. This result contradicts the commonly accepted idea that the sugar conformation at the 3'-end does not influence the dinucleotide stacking behavior[6,7] and demonstrates that the population distribution at the 3'-end is crucial for stacking propensity.

To explain the observed stacking properties of dinucleotides whose 5'-end sugar is N, we suggest that the 5'-N pucker induces geometrical constrains on the sugar phosphate backbone and/or the 3'-end sugar residue that result in an increase and/or stabilization of N-population at the sugar 3'-end. This steering process that has already been reported to occur in the duplex context[22] is therefore effective even at the dinucleotide single strand level, and does not imply the presence of neighboring nucleotide residues. However, if the 3'-end sugar is impeded to adopt an N conformation, the 5'-end N-sugar conformation effect is inoperative to promote stacking.

We thank CNRS, Région Champagne Ardenne and Fondation pour la Recherche Médicale (FDT20130928264) for a doctoral fellowship to J. Jakhlal. Dr C. Gobinet (MEDyC, CNRS UMR 7369) and Dr J.-M. Nuzillard (ICMR, CNRS UMR 7312) are greatfully acknowledged for fruitful discussions on SVD and NMR complexity, respectively.

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