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Synthesis and oligodeoxynucleotide binding properties of pyrrolidinyl peptide nucleic acids bearing prolyl-2-aminocyclopentanecarboxylic acid (ACPC) backbones

Chaturong Suparpprom, Choladda Srisuwannaket, Polkit Sangvanich and Tirayut Vilaivan*

Organic Synthesis Research Unit and Research Centre for Bioorganic Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok 10330, Thailand

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Dedicated to the late Professor Gordon Lowe

Abstract—A series of novel conformationally rigid pyrrolidinyl peptide nucleic acids (PNA) based on D-prolyl-2-aminocyclopentanecarboxylic acid (ACPC) backbones has been synthesized. Investigation of the binding properties of four stereoisomeric PNAs possessing different stereochemistry at the ACPC part with DNA revealed that a precise stereochemistry of the backbone is very important in determining the binding properties. Only the PNA containing (1*S*,2*S*)-ACPC can form a very stable 1:1 complex with the complementary DNA in a sequence-specific manner.

Peptide nucleic acids (PNAs) (**1**) are a novel class of oligonucleotide analogues originally introduced by Nielsen et al. in 1991.[1] The unique ability of PNA to hybridize strongly yet specifically with DNA or RNA, especially under low-salt conditions, leads to a number of novel applications in the field of biotechnology and therapeutics.[2,3] Not surprisingly, much attention has been paid in developing novel PNA analogues with the aim of achieving even better binding properties.[4,5] Inspired by the foldamer concept,[6] we have recently developed novel conformationally rigid PNA analogues with a pyrrolidine core structure in combination with β-amino acid spacers.[7–9] Particularly interesting results were obtained with a PNA bearing a D-aminopyrrolidinecarboxylic acid (DAPC) spacer **2**, which showed preferential binding to complementary DNA over RNA in a highly sequence-specific manner.[8] The failure of the more flexible analogues of **2**, including those similarly carrying positive charges,[9] to show any detectable binding with DNA led us to propose that the appropriate conformation of the spacer amino acid rather than the presence of positive charge is the crucial factor in determining the binding properties of the resulting PNA.

We proposed that *trans*-(1*S*,2*S*)-2-aminocyclopentanecarboxylic acid (*SS*-ACPC) should be a good structural mimic of the protonated DAPC, and should assume a *trans*-configuration, except that it should be uncharged at neutral pH. To test the hypothesis, all four stereoisomeric D-prolyl-ACPC PNAs **3** (Scheme 1) with a C-terminal lysinamide capped T_5 sequence (**3*SS***-T_5, **3*RR***-T_5, **3*SR***-T_5 and **3*RS***-T_5) were synthesized. This was accomplished by solid phase peptide synthesis starting from the Fmoc-protected thymine monomer **4**[7–9] and the Fmoc-protected ACPCs (**5*SS***, **5*RR***, **5*SR***, **5*RS***) (Scheme 2) on an acid labile Rink amide linker as previously described for similar PNAs.[10] The Fmoc-protected acid precursors of the *trans* isomers (**5*SS*** and **5*RR***) were commercially available (Neosystem, Strasbourg, France). The precursors of the *cis*-**5*SR*** and **5*RS*** isomers were readily obtained in diastereomerically enriched form by $NaBH(OAc)_3$ reduction of the (*R*)- and (*S*)-α-methylbenzylenamines of ethyl 2-oxocyclopentanecarboxylate, respectively.[11] These were further purified[12] and transformed into the enantiomerically pure activated monomers **5*SR*** and **5*RS*** according to literature

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* Corresponding author. Tel.: +66 2 2187627; fax: +66 2 2187598; e-mail: vtirayut@chula.ac.th

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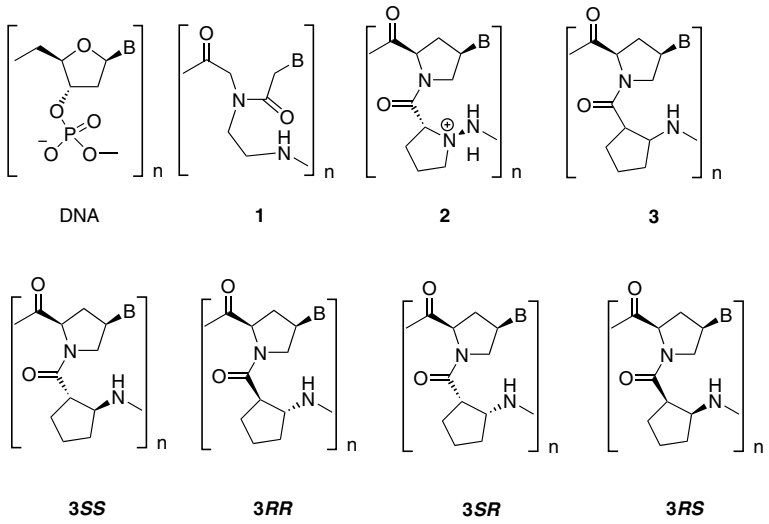
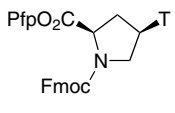


Scheme 1.



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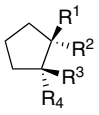

5*SS* R^1 = H; R^2 = CO_2Pfp; R^3 = FmocNH; R^4 = H
5*RR* R^1 = CO_2Pfp; R^2 = H; R^3 = H; R^4 = FmocNH
5*SR* R^1 = H; R^2 = CO_2Pfp; R^3 = H; R^4 = FmocNH
5*RS* R^1 = CO_2Pfp; R^2 = H; R^3 = FmocNH; R^4 = H

Scheme 2.

procedures.[13] The identities of the synthesized PNAs were confirmed by MALDI-TOF mass spectrometry after cleavage from the resin and HPLC purification.[14]

The interaction of all T_5 PNAs with DNA was initially studied by UV titration experiments.[15] Due to the weak interactions between short pieces of PNA and DNA, poly(dA) was chosen as a model of complementary DNA rather than dA_5. In these experiments, poly(dA) was added to the solution of the PNA buffered with sodium phosphate (10 mM) in several aliquots and the UV absorbance at 260 nm (A_{260}) measured after each addition. A significant deviation of the observed UV absorbance at 260 nm from the calculated value due to hypochromism was observed when **3*SS***-T_5 was titrated against poly(dA) (Fig. 1). The plot of the ratio of observed and calculated A_{260} as a function of mole fraction of dA showed an inflection point around 0.5, indicating the formation of a 1:1 PNA·DNA complex. Similar plots for the other three stereoisomeric PNA titrations were essentially flat with the ratio of observed and calculated A_{260} close to unity suggesting that they did not form stable hybrids at the temperature at which the titration was carried out (25 °C).

In order to investigate the nature of the interaction between PNA and DNA in more detail, a longer sequence C-terminal lysinamide capped PNA, **3*SS***-T_{10}, was synthesized (MALDI-TOF MS calcd for M+H$^+$ 3467.6; found 3468.7). UV titration as described above confirmed that **3*SS***-T_{10} forms a well-defined 1:1 complex with dA_{10}. The interaction of **3*SS***-T_{10} and dA_{10} was further investigated using circular dichroism (CD) spectroscopy. The PNA **3*SS***-T_{10} itself had an essentially flat CD spectrum in the region of 200–300 nm while dA_{10} exhibited a weak but characteristic CD signal due to its partial preorganization.[16] The 1:1 mixture of **3*SS***-T_{10} and dA_{10} showed a significantly different CD spectrum from the calculated sum of the CD spectra of the individual components (Fig. 2). The negative bands originally present in the single stranded dA_{10} at 248 and 205 nm intensified upon complexation. A new negative band at 268 nm and a positive band at 284 nm also appeared. This indicates the conformational change induced by the PNA·DNA hybridization. Although CD spectra of the hybrids **3*SS***-T_{10}·dA_{10}, **2**-T_{10}·dA_{10} and poly(dA)·poly(dT) were

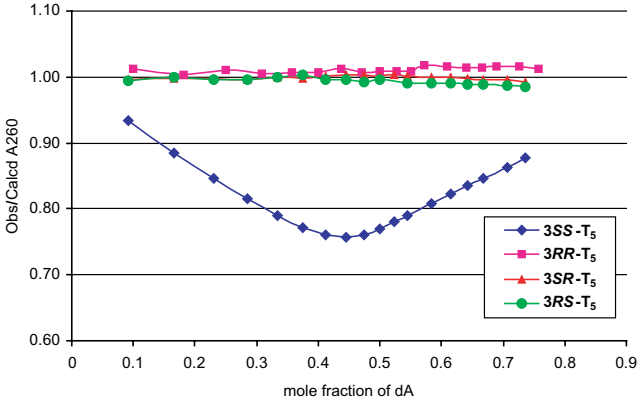

Figure 1. UV titration plot of poly(dA) and all four isomers of PNA **3**-T_5 bearing different stereochemistry of the ACPC spacers (1*S*,2*S*/1*R*,2*R*/1*S*,2*R* and 1*R*,2*S*). The plots show the ratio of observed A_{260}/calculated A_{260} and mole fraction of dA. The concentration of PNA was constant at 2.84 μM. The titrant was dA_{10} (173 μM). Volume correction was applied. Other conditions: 10 mM sodium phosphate buffer, pH 7.0, 25 °C.

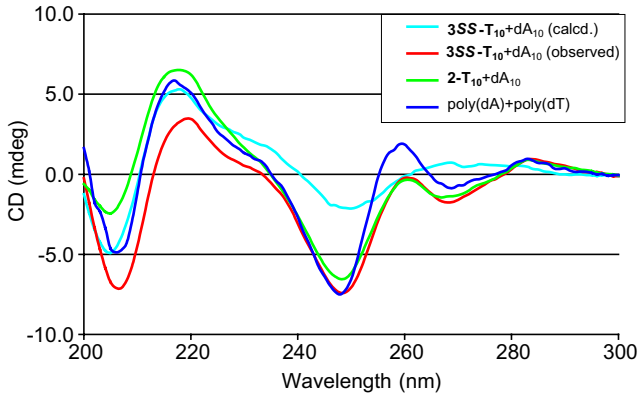

Figure 2. Comparison of the CD spectra of **3*SS***-T_{10}·dA_{10} (calculated and observed CD spectra), **2**-T_{10}·dA_{10} and poly(dA)·poly(dT). The calculated spectrum was obtained from the sum of the two individual components. Concentrations (expressed as dT or dA nucleotide) were kept constant at 10 μM. Other conditions: 10 mM sodium phosphate buffer, pH 7.0, 20 °C.

essentially similar, they also showed slight variations (Fig. 2). This probably reflects the different conformation of the PNA·DNA and DNA·DNA complexes.[17] By varying the PNA:DNA ratio and plotting the CD intensity against the PNA:DNA ratio (data not shown), a 1:1 stoichiometry was established, which is in good agreement with the UV titration experiment above.

Mass spectrometry using soft ionization techniques such as ESI has recently been recognized as a powerful method to detect non-covalent interactions between biological macromolecules, including DNA·DNA and PNA·DNA.[18] In the present study, this technique has been used to study the interaction between **3*SS***-T_{10} and dA_{10}. A multiple charged species, *m/z* 1633.8 Da, corresponding to the charged state $[M-4]^{4-}$ for the complex between **3*SS***-T_{10} and dA_{10} (calculated [(3468.5+3070.1)− 4]/4 = 1633.7) was clearly observed, again confirming the formation of the 1:1 PNA·DNA complex (Fig. 3).

As expected, the absorbance at 260 nm of the 1:1 mixture of **3*SS***-T_{10} and dA_{10} is temperature dependent. Upon heating, melting of the PNA·DNA hybrid was observed as was evident from the sharp increase in the absorbance at 260 nm. However, the melting process was still incomplete at 90 °C therefore it was difficult to determine the exact T_m value. Nevertheless, the T_m value was estimated to be >85 °C indicating that the **3*SS***-T_{10}·dA_{10} hybrid was much more stable than the corresponding DNA·DNA hybrid. The melting was fully reversible and showed only little hysteresis suggesting that binding kinetics were fast. Repeating the T_m experiment between **3*SS***-T_{10} and different oligonucleotide decamers containing one or two mismatch bases resulted in lowered T_m values of the complex by 18–25 °C per mismatch (Fig. 4 and Table 1). The percentage hyperchromicity was also decreased when mismatches were introduced into the DNA strand indicating the reduction of base–base stacking in the resulting hybrids. Judging from these T_m figures, the binding affinity and sequence specificity of PNA **3*SS*** is considerably higher than PNA **1** and **2** for the same T_{10} sequence (Table 1).

The very strong yet highly sequence-specific binding of **3*SS***-T_{10} to its complementary oligodeoxynucleotide is

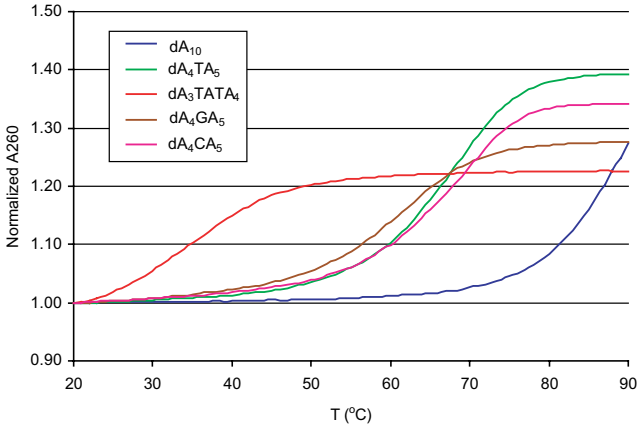

Figure 4. Melting curves of **3*SS***-T_{10} with dA_{10} (perfect match), $d(A_4XA_5)$ (X = T, G, C) (single mismatch) and $d(A_3TATA_4)$ (double mismatch). The T_m was measured at a ratio of PNA:DNA = 1:1, concentration of PNA strand = 1 μM, 10 mM sodium phosphate buffer, pH 7.0, heating rate 0.5 °C/min.

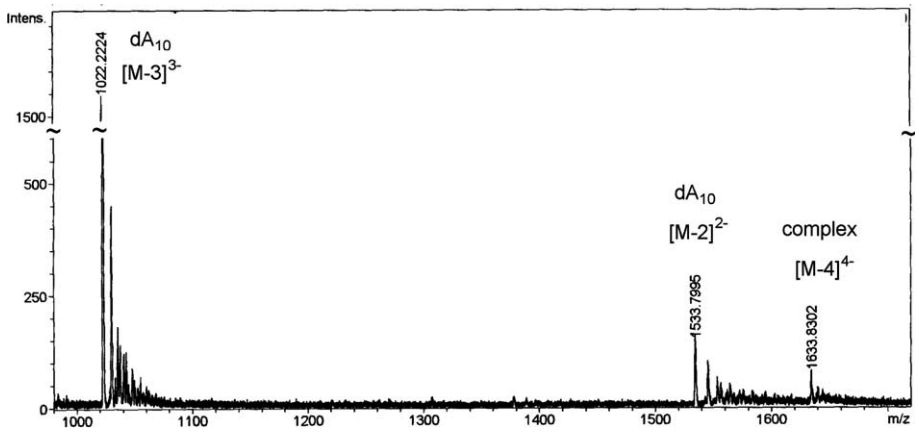

Figure 3. ESI-TOF mass spectrum of a mixture of **3*SS***-T_{10} and dA_{10} at the ratio of 1:1 in negative ion mode.

Table 1. Comparison of the T_m values of PNA·DNA complexes

Entry	PNA	Oligonucleotide	T_m, °C[a] (% hyperchromicity)	Note
1	**3*SS***-T_{10}	dA_{10}	>85	Perfect match
2	**3*SS***-T_{10}	$d(A_4TA_5)$	67 (38)	Single mismatch
2	**3*SS***-T_{10}	$d(A_4CA_5)$	68 (34)	Single mismatch
3	**3*SS***-T_{10}	$d(A_4GA_5)$	61 (27)	Single mismatch
4	**3*SS***-T_{10}	$d(A_3TATA_4)$	34 (23)	Double mismatch
5	**1**-T_{10}	dA_{10}	72[b,c]	Perfect match
6	**1**-T_{10}	$d(A_4GA_5)$	59[b,c]	Single mismatch
7	**2**-T_{10}	dA_{10}	80 (18)[d]	Perfect match
8	**2**-T_{10}	$d(A_4TA_5)$	57 (15)[d]	Single mismatch
9	**2**-T_{10}	$d(A_4TATA_3)$	<30[d]	Double mismatch

[a] Conditions: 10 mM sodium phosphate buffer, pH 7.0; concentration of PNA = DNA ~ 1 μM unless otherwise indicated.
[b] Data from Ref. 1b
[c] Homopyrimidine PNA **1** binds with DNA to form a PNA_2·DNA triplex.
[d] Data from Ref. 8.

quite remarkable. Initially it was thought that in the absence of the positive charge resulting from protonation of the basic ring nitrogen atoms as in **2**, the PNA **3** would bind less strongly to the negatively charged oligonucleotides. The opposite results obtained may be attributed to the more structurally rigid nature of the backbone of **3**, which is held permanently in a favourable conformation to allow its nucleobase to interact with the complementary nucleobase in the DNA strand without the need for protonation. The different stoichiometry of DNA hybrids of PNA **2** and **3** compared to that of Nielsen's PNA[1] **1** also deserves further comments. While **1**-T_{10} exclusively forms the 2:1 complex with dA_{10},[1] **2**-T_{10} and **3**-T_{10} form only 1:1 complexes. The positively charged backbone of **2** is expected to inhibit the formation of a triplex structure since it would preclude the approaching second (also positively charged) PNA strand and would destabilize the triplex so formed. Similar phenomena have been observed in the literature. For example, an analogue of Nielsen-type PNA containing several positively charged lysine moieties in the backbone forms only a duplex with complementary DNA.[19] However, since **3**, with its neutral backbone, also failed to form triplexes, a better explanation would be due to the excessive steric bulk of the backbones of **2** and **3** to triplex formation as compared to Nielsen's PNA **1**.

This work, together with our previous results has confirmed our initial proposal that the appropriate geometry rather than charge of the β-amino acid spacer is essential in determining the binding properties of the resulting PNA.[9] The active role of spacers will be the basis for the design of new PNA systems with even better binding properties.

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References and notes

1. (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1500; (b) Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 1895–1897.
2. Recent reviews: (a) Demidov, V. V. *Drugs Discovery Today* **2002**, *7*, 153–154; (b) Nielsen, P. E. *Curr. Opin. Biotechnol.* **2001**, *12*, 16–20.
3. Recent examples: (a) Chakrabarti, R.; Klibanov, A. M. *J. Am. Chem. Soc.* **2003**, *125*, 12531–12540; (b) Komiyama, M.; Ye, S.; Liang, X.; Yamamoto, Y.; Tomita, T.; Zhou, J.-M.; Aburatani, H. *J. Am. Chem. Soc.* **2003**, *125*, 3758–3762.
4. Recent reviews: (a) Ganesh, K. N.; Nielsen, P. E. *Curr. Org. Chem.* **2000**, *4*, 931–943; (b) Kumar, V. A. *Eur. J. Org. Chem.* **2002**, 2021–2032.
5. Recent examples: (a) Ahn, D.-R.; Mosiman, M.; Leumann, C. J. *J. Org. Chem.* **2003**, *68*, 7693–7699; (b) Huang, Y.; Dey, S.; Zhang, X.; Sonnichsen, F.; Garner, P. *J. Am. Chem. Soc.* **2004**, *126*, 4626–4640; (c) Govindaraju, T.; Gonnade, R. G.; Bhadbhade, M. M.; Kumar, V. A.; Ganesh, K. N. *Org. Lett.* **2003**, *5*, 3013–3016; (d) Kitamatsu, M.; Shigeyasu, M.; Okada, T.; Sisido, M. *Chem. Commun.* **2004**, 1208–1209; (e) Myers, M. C.; Witschi, M. A.; Larionova, N. V.; Franck, J. M.; Haynes, R. D.; Hara, T.; Grajkowski, A.; Appella, D. H. *Org. Lett.* **2003**, *5*, 2695–2698; (f) Samuel, T. H.; Hickman, D. T.; Morral, J.; Beadham, I. G.; Micklefield, J. *Chem. Commun.* **2004**, 516–517.
6. Review: Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *191*, 3219–3232.
7. Vilaivan, T.; Suparpprom, C.; Harnyuttanakorn, P.; Lowe, G. *Tetrahedron Lett.* **2001**, *42*, 5533–5536.
8. Vilaivan, T.; Lowe, G. *J. Am. Chem. Soc.* **2002**, *124*, 9326–9327.
9. Vilaivan, T.; Suparpprom, C.; Duanglaor, P.; Harnyuttanakorn, P.; Lowe, G. *Tetrahedron Lett.* **2003**, *44*, 1663–1666.
10. The synthesis of PNA was carried out on 1.0 μmol scales on Novasyn TGR resin (Novabiochem, Switzerland) [0.20 mmol/g substitution, preloaded with Fmoc-Lys-(Boc)-OH]. The synthetic cycle is as follows: deprotection: 20% piperidine in DMF (1.0 mL, 15 min), wash (DMF), coupling [**4**/HOAt or **5**/HOAt (1:1) in DMF, 4 equiv, 2 h], wash (DMF), capping (10% Ac_2O/DIEA in DMF), wash (DMF). The coupling reaction was monitored by measurement of the amounts of the dibenzofulvene–piperidine adduct released upon deprotection at 264 and 300 nm. After addition of the final residue was complete, the

N-terminal Fmoc group was removed using 20% piperidine in DMF. Average coupling yields in all cases were approximately >95% per step. The PNA was released from the resin by treatment with 95% aqueous trifluoroacetic acid (ca. 1 mL for 10 mg of resin). The TFA was evaporated by a stream of nitrogen and the residue was washed with diethyl ether. The crude PNA was purified by reverse phase HPLC. Sample elution was carried out using a gradient of water–acetonitrile containing 0.1% trifluoroacetic acid (monitoring by UV absorbance at 260 nm). The purified PNA showed only a single peak (>95% purity) when subjected to analysis under the same conditions.

11. Cimarelli, C.; Palmieri, G.; Bartoli, G. *Tetrahedron: Asymmetry* **1994**, *5*, 1455–1458.
12. (a) Fülöp, F. *Chem. Rev.* **2001**, *101*, 2181–2204; (b) Nöteberg, N.; Brånalt, J.; Kvarnström, I.; Classon, B.; Samuelsson, B.; Nillroth, U.; Danielson, H.; Karlén, A.; Hallberg, A. *Tetrahedron* **1997**, *53*, 7975–7984; (c) Szakonyi, Z.; Fülöp, F.; Bernáth, G.; Török, G.; Péter, A. *Tetrahedron: Asymmetry* **1998**, *9*, 993–999.
13. Dener, J. M.; Fantauzzi, P. P.; Kshirsagar, T. A.; Kelly, D. E.; Wolfe, A. B. *Org. Proc. Res. Dev.* **2001**, *5*, 445–449.
14. MALDI-TOF data of PNA **3** (CCA matrix): **3**-H-T_5-LysNH_2 calcd for $M+H^+$ 1806.9; found **3*SS***-T_5 1807.0, **3*RR***-T_5 1806.8, **3*SR***-T_5 1806.9, **3*RS***-T_5 1806.6.
15. Lowe, G.; Vilaivan, T.; Westwell, M. S. *Bioorg. Chem.* **1997**, *25*, 321–329.
16. Steely, H. T.; Gray, D. M.; Ratliff, R. L. *Nucleic Acids Res.* **1986**, *14*, 10071–10090.
17. Johnson, K. H.; Gray, D. M.; Sutherland, J. C. *Nucleic Acids Res.* **1991**, *19*, 2275–2280.
18. Review: Hofstadter, S.; Griffey, R. H. *Chem. Rev.* **2001**, *101*, 377–390.
19. Zhou, P.; Wang, M.; Du, L.; Fisher, G. W.; Waggoner, A.; Ly, D. H. *J. Am. Chem. Soc.* **2003**, *125*, 6878–6879.