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Convenient synthesis and cyclization of dimeric abasic PNA

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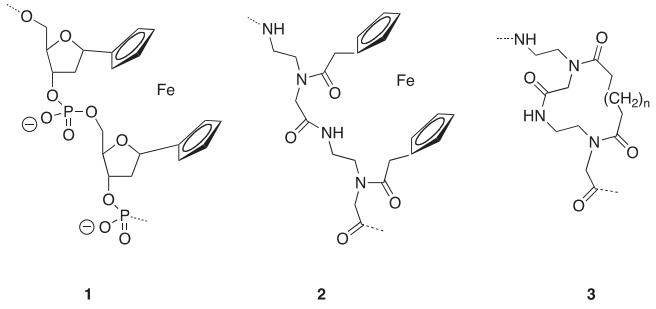
ABSTRACT

The synthesis of an abasic PNA-based dimer block has been achieved. An alkyl chain stapling the two base sites conformationally restricts the PNA backbone, and serves as an example of preorganization by direct base site linkage. Other possible examples of this strategy, such as enforced stacking of adjacent intrastrand bases to give base pair step analogs, are put forward.

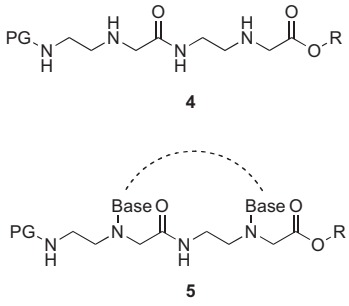
DNA analogs have been reported with almost every imaginable structural substitution.[1,2] Different base pairs, backbones, and sugar types are well known and in a few cases have led to marketable drugs.[3] What is retained is some mechanism to limit the conformational flexibility. One feature of natural double-helical deoxyribonucleic acids that leads to a predictable but variable structure is intrastrand base stacking.[4,5] In DNA, some steps, such as 5′-TpdA-3′, are highly flexible, while 5′-dApdA-3′ steps adopt the canonical B-DNA conformation.[6] Different preferences for the step parameters twist, roll, tilt, rise, slide, and shift lead to the differences in local helical parameters seen in structural studies and to differences in flexibility.[7] While a great deal of research has focused on nucleic acid base pair analogs[2,8–10] we are unaware of studies directed toward the design and synthesis of intrastrand base pair stacking analogs.[11,12] In our opinion, what is crucial for a base pair step is that it forms a pseudo-ring, preorganizing the backbone in a helical conformation with flexibility in at least some step parameters to accommodate adjacent sequences. In native DNA, hydrophobic and dispersion forces act like a pseudoatom (of the type one can use in molecular modeling) in the structure to keep adjacent bases stacked. There are other conceivable alternatives to these forces.

Examples of molecules that illustrate the idea of a base pair step stacking analog are **1**–**3,** Figure 1. Since the distance between cyclopentadienyl rings in ferrocene is 3.32 Å while the distance between bases in B-DNA is 3.4 Å (a similarity first pointed out by Inouye and co-workers[13,14]), ferrocenes make attractive base pair step analogs, either for deoxyribose-phosphodiester backbones or peptide nucleic acid backbones, with the iron atom acting as the pseudoatom.



1 2 3

Figure 1. DNA/Fc conjugate **1**, PNA/Fc conjugate **2**, and PNA/alkyl **3**.



4

5

Figure 2. PNA scaffolding **4** and desired linked bases **5** PG = protecting group.

However, direct attachment of ferrocene to a deoxyribose C1′ leads to acid instability and epimerization at the anomeric center mediated by ferrocene's strong stabilization of adjacent carbocations (unpublished results, J. W. Suggs). Alternatively, a peptide

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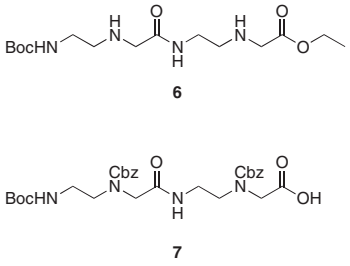


6 7

Figure 3. Desired backbone material **6** and the literature precursor **7**.

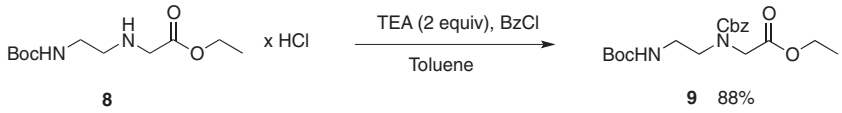
nucleic acid backbone can be used as in **2**, which solves any epimerization problems and introduces no new stereogenic centers. The simplest ferrocene base pair step analog would contain a Fc-CH_2CONR_2 group. Since ferrocenes with $FcCH_2COX$ groups undergo autoxidation at CH_2,[15–17] compounds with this kind of step would be air sensitive. Thus, the most direct realization of a nonreactive group capable of preorganizing a nucleic acid chain into a helical conformation would be an alkyl chain as depicted in **3**. Such a strategy has been used to preorganize peptide backbones into an α-helical conformation (stapled peptides).[18] The generalized PNA (peptide nucleic acid) architecture for a base pair step analog is shown in Figure 2 (idealized as **5**). Note in this initial realization the linkage functions only to constrain the conformation of one chain by creating a ring, not to interact with bases in the adjacent strand. In this way it mimics the nucleic acid universal bases.[19]

A Boc/Z protecting group strategy was chosen for **4** due to its literature precedence, chemical orthogonality, and applicability to PNA automated synthesis.[20] We therefore chose **6** as the backbone target, which has the literature precedent, 7.[21]

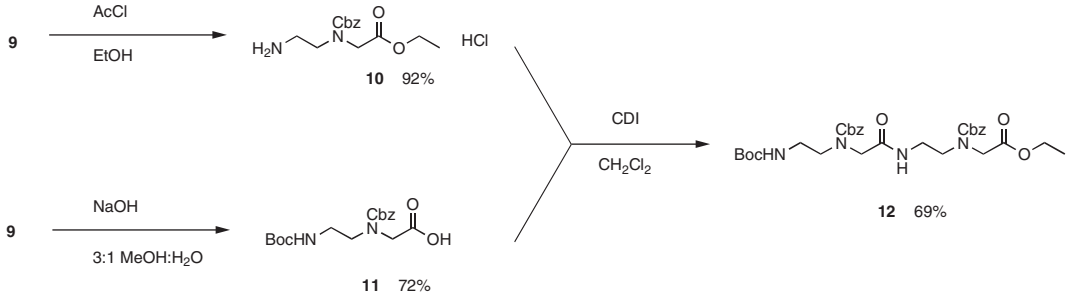
In our experience, the published procedure for **7**[21] Figure 3 was difficult to scale, required careful pH control, and was low yielding. We have discovered conditions that provide multigram quantities and are scalable and economical. The key Boc/*Z* monomer **9** was made from hydrochloride salt **8**[22] in 88% (Scheme 1).

With bulk **9** in hand, Boc deprotection using AcCl/EtOH[23] directly afforded hydrochloride **10** while hydrolysis afforded free acid **11**. Conveniently, both monomeric compounds were obtained as white solid powders (Scheme 2).

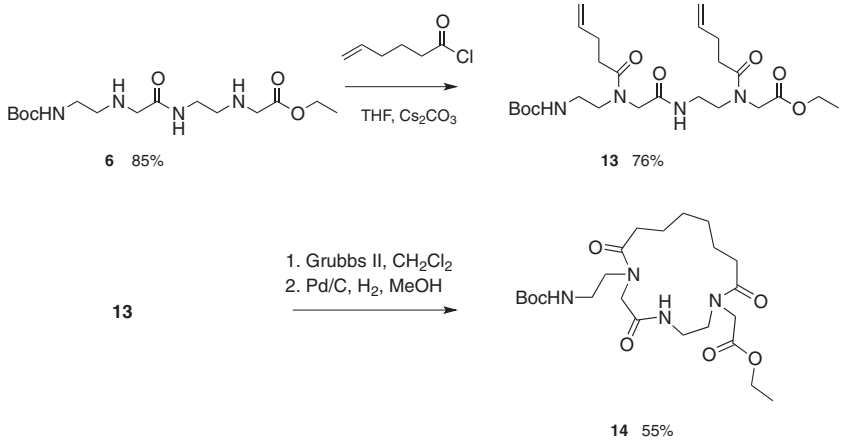
As a major goal was operational ease, we wished to avoid any use of polar aprotic solvents typically employed when attempting peptide coupling directly with amine salts. Carbonyl diimidazole, used industrially for peptide couplings[24] smoothly delivered the desired dimer **12** in good yield. The simplicity of the reaction is notable: solid hydrochloride salt was added in a single portion to a solution of activated **11** and allowed to stir overnight. Catalytic



Scheme 1. Preparation of fully protected monomer **9**.



Scheme 2. Route to fully protected dimer **12**.



Scheme 3. Preparation of desired alkyl-ring macrocycle **14**.

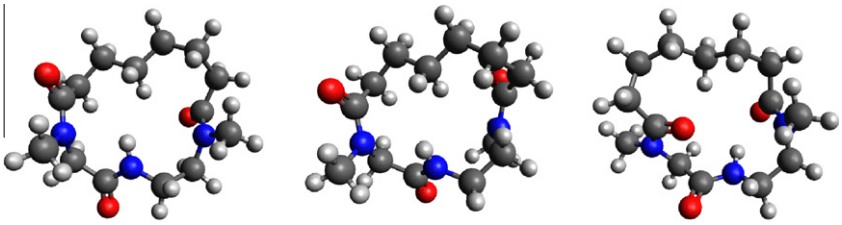


Figure 4. Calculated lowest energy conformers of **14** (ΔE kcal/mol 0.00, 2.77, and 3.69 for **15**, **16**, and **17**, respectively).

hydrogenation of **12** afforded deprotected **6** in 85% yield, representing our desired scaffolding **4**.

To illustrate linking, we reacted **6** with 4-pentenoic acid chloride in THF/$CsCO_3$ smoothly affording diacylated **13** in 76% yield. Treatment of **13** with Grubbs' II catalyst followed by catalytic hydrogenation afforded macrocycle **14** in 55% over two steps. HPLC provided an analytical sample of **14** (single peak, 97% purity by UV trace integration) (Scheme 3).

As was expected due to restricted rotation about tertiary amide bonds,[25] the NMR spectra of **14** were obtained as a complex mixture of conformers. A computational investigation of the ring system was performed by running geometry optimizations on all possible cis/trans amide conformers at the B3LYP/6-31G** level of theory (details in Supplementary data). The 2D COSY spectrum clearly revealed that at least three conformers were present in significant amounts, and the computational results indeed indicated that four of the possible eight conformers are within only 5 kcal/mol. The 3 calculated lowest energy conformations of **14** are shown in Figure 4.

In summary an expedient synthesis of scaffolding **6** and a simple example of a 'universal' base-pair step analog **14** have been achieved. It is notable that every reaction toward preparing **14** is either catalytic or uses exceedingly common and inexpensive reagents. Every step toward backbone **6** is high yielding and operationally simple. This allows easy access to research quantities of an abasic PNA dimer.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.10.032.

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