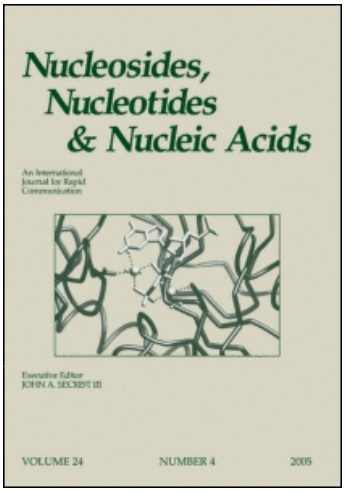

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Progress Towards a Submonomer Synthesis of Peptide Nucleic Acid

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Progress Towards a Submonomer Synthesis of Peptide Nucleic Acid

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ABSTRACT

We report recent developments in the optimization of a submonomer synthesis of peptide nucleic acid based on the Fukuyama-Mitsunobu reaction. The key steps in the submonomer synthesis are the installation of an appropriately protected 2-aminoethyl group on the α-nitrogen of an amino acid and its subsequent acylation with a protected nucleobase derivative. The aggressive alkylation conditions require a scheme of maximal protection for the nucleobases and that is proposed herein for the pyrimidines.

Our group[1] and others[2–6] have been pursuing a new synthetic approach to PNA based on the stepwise assembly of PNA monomers, towards oligomers, on solid supports. This approach promises a reduction in the effort required to synthesize modified PNAs due to the potential for automation, but is not without significant challenges especially with regard to reaction efficiencies and a comprehensive and effective protecting group strategy.

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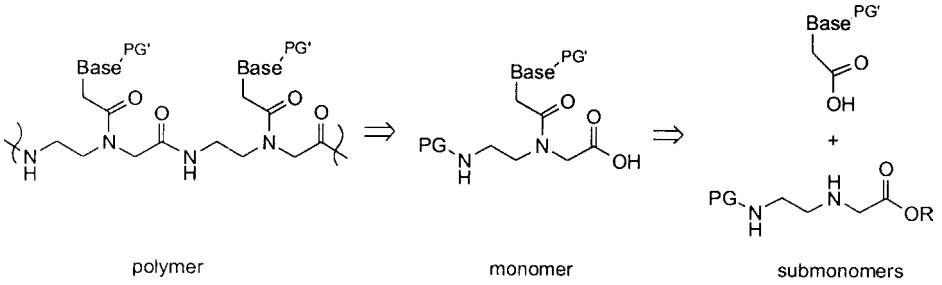
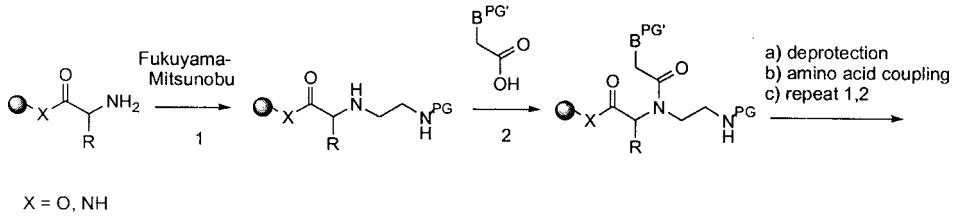


Scheme 1.

The conventional synthetic strategy for PNA is illustrated in Sch. 1, wherein oligomers are assembled by solid-phase peptide synthesis from individual monomers. These monomers are most commonly accessed by the condensation of an appropriately protected nucleobase acetic acid derivative with a protected 2-aminoethylglycinate derivative. Depending on the protecting group scheme, the latter component may be synthesized from the reaction of methyl bromoacetate with mono-protected ethylenediamine, for example. The common routes to modified PNA mirror the traditional synthesis wherein the monomer is synthesized from the corresponding modified components (nucleobase or backbone). The introduction of an α-amino acid with a pre-existing stereocenter may be introduced via reductive amination[7] or Fukuyama amine synthesis.[8] To make diverse oligomers with an large selection of amino acids, substituted ethylamine components and nucleobases would be extremely tedious by the conventional route due to the sheer number of combinations.

As outlined in Sch. 2, the submonomer approach that we have pursued hinges on the Fukuyama-Mitsunobu alkylation of an amino acid, step 1. Subsequent acylation of the secondary amine with a protected nucleobase derivative, step 2, then produces the resin-bound PNA monomer. We have endeavored to optimize each step starting with glycine supported on an insoluble support. We have chosen to do the evaluation of this chemistry on Wang resin, since it is conveniently cleaved by TFA and reactions can be monitored directly by ^{1}H NMR. In general, aliquots of resin were taken at regular time intervals, thoroughly rinsed, dried in vacuo, and cleaved with D-TFA directly into an NMR tube and observed.

This Fukuyama-Mitsunobu transformation is a sequence of three distinct reactions. A primary amine is converted to a sulfonamide derivative that possesses an acidic hydrogen which can then participate in the Mitsunobu alkylation with an alcohol. The final step is a desulfonylation that furnishes a secondary amine. We



Scheme 2. Proposed submonomer assembly of PNA.

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have previously reported that the installation of the *o*-nitrobenzenesulfonyl (nosyl) group proceeds rapidly under the conditions employed but also produces a substantial amount of the di(nosyl) glycine. This is significant since the di(nosyl) glycine would not be competent in the Mitsunobu reaction. However, one nosyl group may be selectively removed by a brief piperidine treatment giving quantitative conversion to nosylglycine. For the second step in the sequence, several amino-protected ethanolamines were evaluated (e.g., 2-azidoethanol, N-Boc-ethanolamine) and the dimethoxytrityl (DMT) group was chosen for N-protection due to its compatibility with the Wang resin and stability to all of the reaction conditions it would encounter. Early solution-phase studies on the Mitsunobu reaction indicated that it was necessary to use Tsunoda's modified conditions (TMAD, PBu_3)[9] with the addition of a base in order for reactions to achieve acceptably high levels of conversion within reasonable times. We were interested to study this phenomenon more closely and chose to compare standard Mitsunobu conditions to Tsunoda's modified conditions with or without base, Table 1. For this study, we prepared a carbon-based isostere of N-DMT-ethanolamine to address concerns about possible side reactions due to the lability of the DMT-group. Based on this limited study, we have not observed a general acceleration of the reaction by inclusion of a base. As expected based on the most commonly encountered conditions in the chemical literature, the most reliable reactions took place with the combination of an azodicarboxylate with triphenylphosphine. It is noteworthy, that in each case the N-DMT-ethanolamine **C** reacts more slowly than the isosteric alcohol **B**. Although not previously reported, to our knowledge, the use of an azodicarboxamide (TMAD) with triphenylphospine also produces satisfactory results but requires slightly longer reaction times. Conversely, the use of a more basic phosphine (PBu_3) with an azodicarboxylate reagent does not confer any advantage.

Since aggressive alkylating conditions are used to install the protected 2-aminoethyl group, protection of the nucleobases presents a challenging problem. Starting with the thymine submonomer, we observed substantial N^3-modification under the Mitsunobu reaction conditions used. We used this reaction to install a 2-trimethylsilylethyl- group, however it was not removed under any conventional conditions. Our choice of protecting group subsequently became the *p*-methoxybenzyl (PMB) group which can be introduced and removed readily. Cytosine poses more of a challenge to protect, since the N^4-CBz derivative still modified under Mitsunobu conditions employed. Initially, the acid-labile CBz was combined with the PMB to replace both N^4-protons in order to have straightforward deprotection conditions. To our surprise, the CBz is removed more easily than the PMB group under the conditions employed. As well, the CBz group is unstable towards base hydrolysis in the di-protected nucleobase. Next, N^4-(di-PMB) cytosine was prepared (Sch. 3) and evaluated. Although the PMB groups were stable to base hydrolysis, attempts to form the carboxylic acid fluoride failed.[a] We reasoned that an electron withdrawing group

[a]We previously found that common peptide coupling reagents such as PyBroP, PyBOP, HBTU or HATU failed to produce satisfactory coupling of the nucleobase submonomer to resin-bound protected 2-aminoethylglycinate derivatives. However, use of the acid fluoride produced quantitative coupling within 15 minutes for the thymine submonomer.

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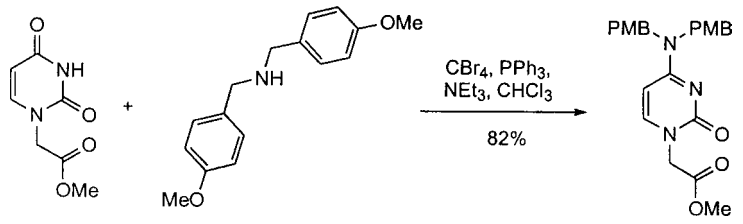
Table 1. Effect of base on the Mitsunobu reaction.

					Chemical Yield (%)[e]		
Entry	Alcohol[a]	Base[b]	Azo compound[c]	Phosphine[d]	20 min	40 min	60 min
1	A	DIEA	DIAD	TPP	100	–	–
2	B	DIEA	DIAD	TPP	100	–	–
3	C	DIEA	DIAD	TPP	74.6	100	–
4	A	DIEA	DIAD	TBP	74.6	95.2	94.3
5	B	DIEA	DIAD	TBP	45.7	55.6	59.5
6	A	DIEA	TMAD	TPP	44.6	76.9	94.4
7	B	DIEA	TMAD	TPP	17.4	100	–
8	A	DIEA	TMAD	TBP	8.6	21.7	30.1
9	B	DIEA	TMAD	TBP	32.3	100	–
10	C	DIEA	TMAD	TBP	46.3	76.9	82.6
11	A	–	DIAD	TPP	100	–	–
12	B	–	DIAD	TPP	100	–	–
13	C	–	DIAD	TPP	91.7	100	–
14	A	–	DIAD	TBP	88.5	92.6	99
15	B	–	DIAD	TBP	68.5	100	–
16	A	–	TMAD	TPP	55.2	94.3	94.4
17	B	–	TMAD	TPP	26.6	100	–
18	A	–	TMAD	TBP	6.4	18.3	27.8
19	B	–	TMAD	TBP	74.6	100	–
20	C	–	TMAD	TBP	0	0	0

[a]A = 1-propanol, B = 4,4-bis(4-methoxyphenyl)-4-phenylbutan-1-ol, C = N-DMT-ethanolamine.
[b]DIEA = di-isopropylethylamine.
[c]DIAD = di-isopropylazodiacarboxylate, TMAD = tetramethylazodicarbonamide.
[d]TPP = triphenylphosphine, TBP = tributylphosphine.
[e]Reaction conditions: 3 eq. alcohol, azo reagent and phosphine, each at 0.25 M in dry THF using N-nosylglycine Wang resin, according to reference 1. Base was either included (10 eq.) or not, as indicated. Chemical yields were determined by ^{1}H NMR.

should overcome this difficulty and prepared the (N^4-PMB, N^4-nosyl) cytosine (Fig. 1) by a similar route. The carboxylic acid derivative of this compound smoothly underwent conversion to the acid fluoride by reaction with cyanuric fluoride.[10]



Scheme 3.

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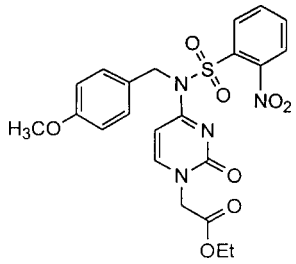

Figure 1. Structure of the di-protected cytosine submonomer precursor.

It is expected that this derivative will be an appropriate cytosine submonomer in our strategy with the nosyl group acting as temporary protection. After installation of this submonomer, the nosyl group is expected to be removed by subsequent Fukuyama-Mitsunobu cycles leaving only PMB protection on cytosine. With the difficulties experienced in acylation or carbamylation N^4-PMB cytosine, it is speculated that this should afford adequate protection during the electrophilic conditions encountered in the submonomer synthesis cycle.

We are continuing to investigate the suitability for the PMB/nosyl protecting group strategy for the submonomer synthesis of pyrimidine PNA and these results will be forthcoming.

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