DOI: 10.1002/chem.201201337

Expanding the Scope of PNA-Encoded Synthesis (PES): Mtt-Protected PNA Fully Orthogonal to Fmoc Chemistry and a Broad Array of Robust Diversity-Generating Reactions

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Abstract: Nucleic acid-encoded libraries are emerging as an attractive and highly miniaturized format for the rapid identification of protein ligands. An important criterion in the synthesis of nucleic acid encoded libraries is the scope of reactions that can be used to introduce molecular diversity and devise divergent pathways for diversity-oriented synthesis (DOS). To date, the protecting group strategies that have been used in peptide nucleic acid (PNA) encoded synthesis (PES) have limited the choice of reactions used in the library synthesis to just a few prototypes. Herein, we describe the preparation of PNA monomers with a protecting group combination (Mtt/Boc) that is orthogonal to Fmoc-based synthesis and compatible with a large palette of reactions that have been productively used in DOS (palladium cross-couplings, metathesis, reductive amination, amidation, heterocycle formation, nucleophilic addition, conjugate additions, Pictet–Spengler cyclization). We incorporate γ-modifications in the PNA backbone that are known to enhance hybridization and solubility. We demonstrate the robustness of this strategy with a library synthesis that is characterized by MALDI MS analysis at every step.

Keywords: combinatorial chemistry · drug discovery · multicomponent reactions · protecting groups · synthetic methods

Introduction

The discovery of small molecules that perturb a given biological pathway by binding to a specific target is at the core of chemical biology and drug discovery. Whereas high-throughput screening (HTS) approaches have proven effective, there remains a need to accelerate the discovery of bioactive compounds and reduce the overall cost.[1] Encoding technologies based on nucleic acid tags are emerging as a potential way to achieve this goal.[2] Nucleic acid encoding platforms have important assets: 1) the nucleic acid tag enables decoding of selected compounds from large mixtures; 2) it offers a highly miniaturized format (screens are typically carried out with just a few micrograms of protein without the need for complex robotics); 3) it does not require intrinsic knowledge of the target (as opposed to displacement assays); 4) amplification of the tags followed by translation into the encoded molecules enables iterative cycles of selection/amplification. Two nucleic acid platforms have been successfully utilized for the encoding: DNA[3] and PNA[4] (peptide nucleic acid[5]). Whereas the general idea of encoding the synthesis of small molecule libraries with DNA was advanced at the onset of combinatorial chemistry,[6] its practical implementation was not straightforward. Breakthroughs in the area have come from innovative strategies to achieve chemical transformations of suitably derivatized DNA by using templated chemistry or sorting methods[7] (see also references cited in ref. [2]). Concurrently, PNA-encoded synthesis (PES) was developed[2d,8] (see also references cited in ref. [3]) based on the premise that its peptide-based chemistry is more robust than the phosphoramidite chemistry of DNA and should be compatible with classical split and mix combinatorial synthesis (Figure 1). Multiple libraries have now been reported with PES. The libraries can either be used directly for affinity selection, or displayed on DNA arrays or on DNA-templates in solution. Either format also offers the possibility of combining libraries of fragments.[4a,e,f] Ultimately, an important criterion for nucleic acid encoded libraries will be the quality and the scope of the chemistry available to access a relevant diversity space. To date, the protecting group strategies that have been used in PES have limited the scope of reactions used in the library synthesis to just a few prototypes. Herein, we describe the preparation of PNA monomers with a protecting group combination (Mtt/Boc) that is orthogonal to Fmoc-based synthesis and is compatible with a broad array of complexity-building reactions.

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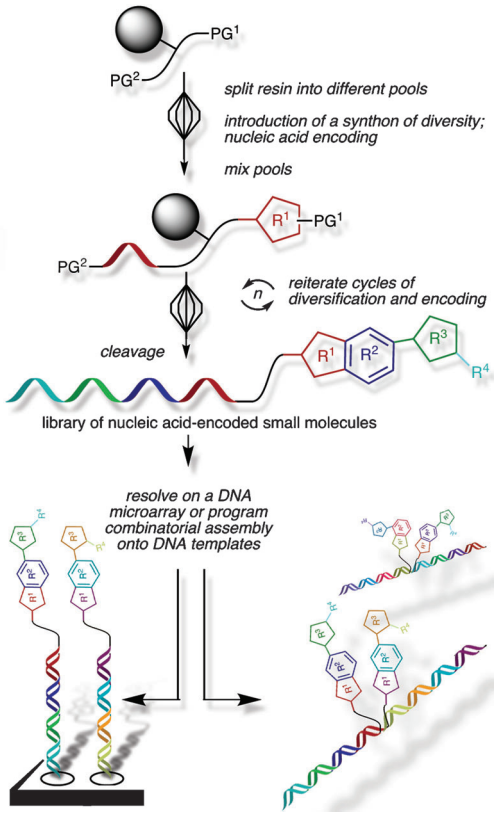
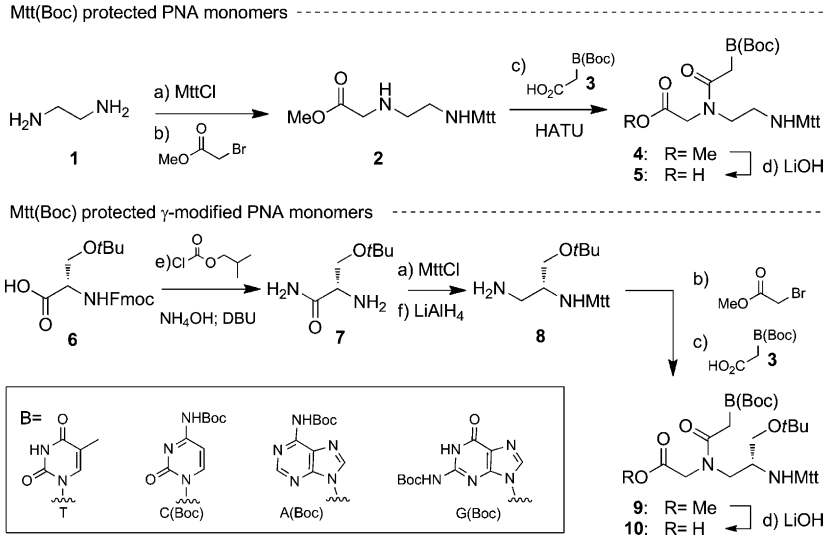


Figure 1. PNA-encoded synthesis (PES) by split and mix and reformatting of the library into a spatially addressable microarray or combinatorial display of fragments.

Results and Discussion

Several strategies have been reported for PES using commercially available Fmoc-protected PNA monomers[4a,b,d,e,8a,b] (Bhoc protected nucleobases), Dde-protected monomers[4h–l] (trityl protected nucleobases), and Azoc-protected monomers (Boc-protected nucleobases).[8c] Nonetheless, with the exception of one example,[4f] these strategies have been restrained to peptide libraries and the orthogonality of the protecting group does limit a broader scope. For example, the sensitivity of Fmoc to basic conditions is a severe limitation; the sensitivity of Dde to nucleophiles is restrictive, and the rapid reaction of Azoc with phosphane precludes most palladium-catalyzed reactions. To this end, we envisioned using a 4-methyl trityl (Mtt)[9] group, which we anticipated to be compatible with the majority of robust reactions that have proven to be useful in diversity oriented synthesis (DOS). Furthermore, the Mtt group is well-known to be orthogonal to Fmoc and should allow the cosynthesis with Fmoc-protected building blocks.[10] We had established that Boc-protected nucleobases are stable to 1% trifluoroacetic acid (TFA)[8c] and, hence, should be compatible with Mtt chemistry and Rink resin. Monomethoxy trityl (Mmt) protected PNA monomers in combination with base-labile acyl groups were previously reported to offer a synthetic strategy similar to standard oligonucleotide synthesis conditions in the preparation of PNA–DNA chimera.[11] However, amino-Mmt groups are extremely labile and require special precautions that are not practical for PES. The synthesis of Mtt-protected monomers is shown in Scheme 1. Monoprotection of ethylene diamine with Mtt-Cl followed by alkylation with methyl bromoacetate afforded the backbone **2** in good yield with simple scalable procedures. Acylation with a range of Boc-protected nucleobases followed by saponification afforded four different monomers (**5A**, **5C**, **5G**, and **5T**) in good yield.

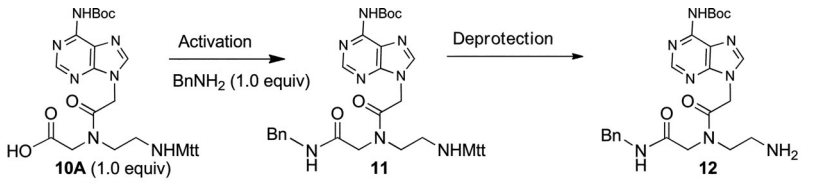
One issue with PNA-encoded libraries is the poor solubility of the PNA oligomers. This led us to explore the incorporation of modified PNA containing a lysine or arginine residue in place of the glycine of the PNA backbone[8a] (modification at the α position of the PNA backbone). Recent studies have highlighted the benefits of modifications at the γ position of the PNA backbone.[12] Such modifications confer



Scheme 1. Synthesis of Mtt(Boc)-protected monomers **5** (A, C, G, T) and **10** (A, C, G, T). Reagents and conditions: a) MttCl (1.0 equiv), Py, CH_2Cl_2, 4 h, 97%; b) Et_3N (1.0 equiv), $BrCH_2CO_2Me$ (1.1 equiv), CH_2Cl_2, 3 h, 70%; c) **3T**, **3C**, **3A**, or **3G** (1.5 equiv), DIPEA (1.8 equiv), 2,6-lutidine (1.5 equiv), HATU (1.4 equiv), DMF, 12 h, 83% for **4C**, 95% for **4T**, 88% for **4G** and 85% for **4A**; d) LiOH (4.0 equiv), dioxane/H_2O (3:1), 2 h, 82% for **5C**, 83% for **5T**, 75% for **5G** and 78% for **5A**; e) NMM (1.0 equiv), C_4H_9COCl (1.0 equiv), DME, then NH_4OH (4.0 equiv), 4 h; DBU (0.4 equiv), CH_2Cl_2, 6 h, 80%; f) LAH (7.0 equiv), Et_2O, 48 h, 73%; b) Et_3N (1.0 equiv), $BrCH_2CO_2Me$ (1.1 equiv), CH_2Cl_2, 3 h, 68%; c) **3T**, **3C**, **3A** or **3G** (1.5 equiv), DIPEA (1.8 equiv), 2,6-lutidine (1.5 equiv), HATU (1.4 equiv), DMF, 12 h, 75% for **9C**, 98% for **9T**, 90% for **9G**, and 78% for **9A**; d) LiOH (4.0 equiv), dioxane/H_2O (3:1), 2 h, 82% for **10C**, 80% for **10T**, 86% for **10G**, and 87% for **10A**. DIPEA = *N*,*N*-diisopropylethylamine; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF = dimethylformamide; HATU = 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium; Mtt = 4-methyltrityl; NMM = *N*-methylmorpholine; Py = pyridine.

a helicity to the PNA, which further enhances its affinity and specificity while providing a handle for solubilizing groups (γ-modification originating from L-amino acids promote right handedness).[12c] To this end, we set out to synthesize the serine-modified PNA monomers **10** (Scheme 1). Starting from Fmoc-Ser(*t*Bu)-OH, the serine amide **7** was obtained in excellent yield. Amine protection followed by reduction of the amide with lithium aluminum hydride provided the required Mtt-protected diamine. This strategy was selected because it is known to be resistant to epimerization.[13] The remaining alkylation, acylation, and hydrolysis following the same procedures used for the unmodified PNA monomers afforded equally efficiently modified monomers **10A**, **10C**, **10G**, and **10T**.

With the PNA monomers in hand, we next turned our attention to the synthesis of oligomers and to the optimization of the activation and deprotection steps. Classic carboxylic activation with uronium (HATU, HBTU, HCTU, TNTU, TSTU), phosphonium (BOP) and carbodiimide (DIC) conditions were evaluated. To assess the relative efficiency of the reaction under conditions that could be readily adapted to automated synthesis, the reaction yields were compared by LC/MS analysis of crude reaction mixtures by using one equivalent each of monomer **10A**, activator, and amine (Scheme 2). Gratifyingly, the potential side reaction leading to a six-membered lactam was not observed under any conditions. The best yields were observed with the succinimide esters (TSTU and TNTU, 71 and 82% yield, respectively). Assessing the next



Conditions	Yield (%)	Conditions	Result
HATU, *i*Pr$_2$EtN, 2,6 lut, DMF 23 °C, 30 min	19	1% TFA in CH_2Cl_2	quant., <5 min
HATU, *i*Pr$_2$EtN, 2,6 lut, DMF 50 °C, 30 min	20	HFIP	quant., <10 min
HBTU, *i*Pr$_2$EtN, 2,6 lut, DMF 23 °C, 30 min	53	HFIP : CH_2Cl_2 (1:1)	quant., <10 min
BOP, *i*Pr$_2$EtN, 2,6 lut, DMF 23 °C, 30 min	67	10% PivOH in CH_2Cl_2	>10%, 5 min
HATU, *i*Pr$_2$EtN, 2,6 lut, DMF 23 °C, 30 min	48	HFIP : DCE, HOBt (5.0 equiv)	quant., <5 min
TNTU, *i*Pr$_2$EtN, 2,6 lut, DMF 23 °C, 30 min	82		
TSTU, *i*Pr$_2$EtN, 2,6 lut, DMF 23 °C, 30 min	71		
DIC, DMAP (0.1 equiv), DMF 23 °C, 30 min	18		

Scheme 2. Optimization of coupling and deprotection conditions.

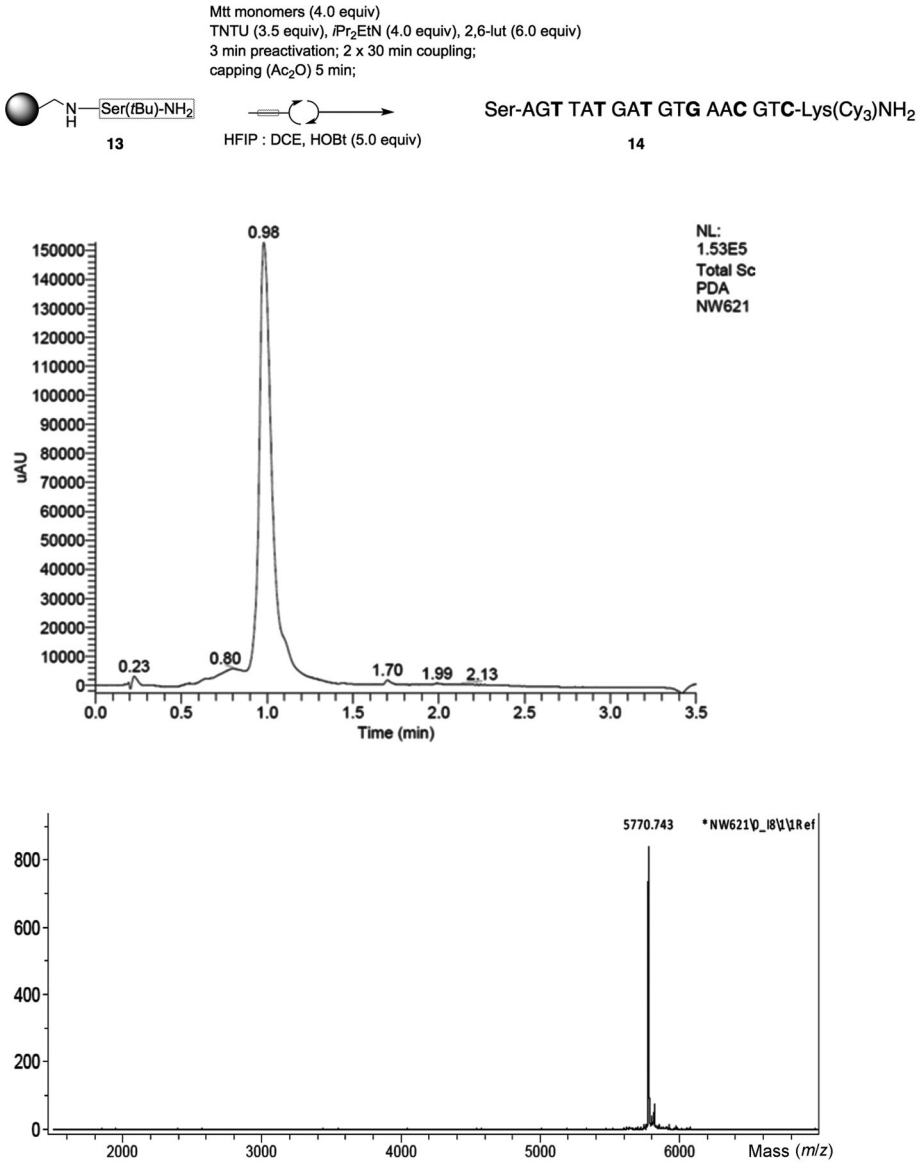


Figure 2. Automated synthesis of an 18 mer PNA. Analysis of the crude cleavage product by LC/MS (top) and MALDI MS analysis (bottom). Bold letters denote serine-modified residues.

step in the sequence, the deprotection of Mtt was complete in under 5 min by using either 1% TFA in dichloromethane or in 10 min with neat hexafluoroisopropanol (HFIP). However, neither conditions were deemed ideal; TFA is corrosive and is not recommended for use in automated synthesizers, whereas HFIP does not swell well the resin. Diluting the HFIP with dichloroethane gave comparably fast reaction while providing a better resin swelling profile. Finally, to keep the amine resulting from the deprotection protonated as a salt, a small excess of 1-hydroxybenzotriazole (HOBt) was included in the deprotection solution. This proved to be beneficial for the Mtt deprotection as well, affording complete deprotection in less than 5 min. We then used the best conditions identified in the solid-phase synthesis of oligomers containing one modified PNA monomer per codon. As shown in Figure 2, the crude cleavage of an 18-mer PNA showed a single major peak, corresponding to the desired oligomer, attesting to the efficiency of the chemistry.

We next investigated the impact of serine-modified PNA in the context of microarray hybridization. To this end, microarrays containing combinatorial permutations of four sets of codons representing 625 combinations of 14 mers were used. The arrays are particularly informative with regards to sequence selectivity because, for any given sequence on the array, there are 19 other sequences that share three out of the four codons. We chose a sequence that had previously been identified as particularly promiscuous due to a long stretch of GC nucleotides and measured the fluorescent intensity for the perfect match as well as the brightest mismatch starting at 2 nM with two-fold dilutions (i.e., 1, 0.5, 0.25 and 0.125 nM, and 62.5, 31.2, and 16 pM). As shown in Figure 3, the modified PNA was three to five times more intense at a given concentration, resulting in more sensitive detection threshold (31.2 pM) relative to unmodified PNA (125 pM). More importantly, the modified PNA showed a

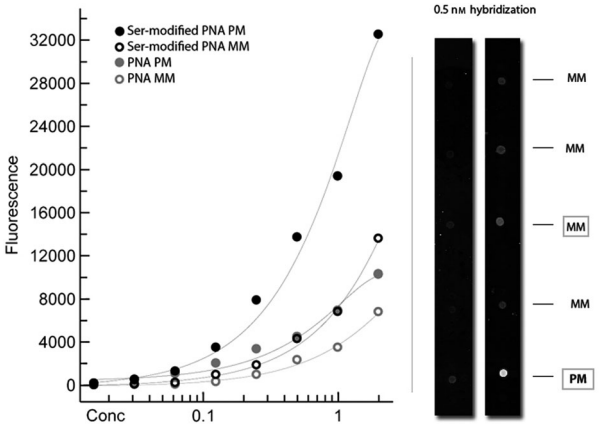
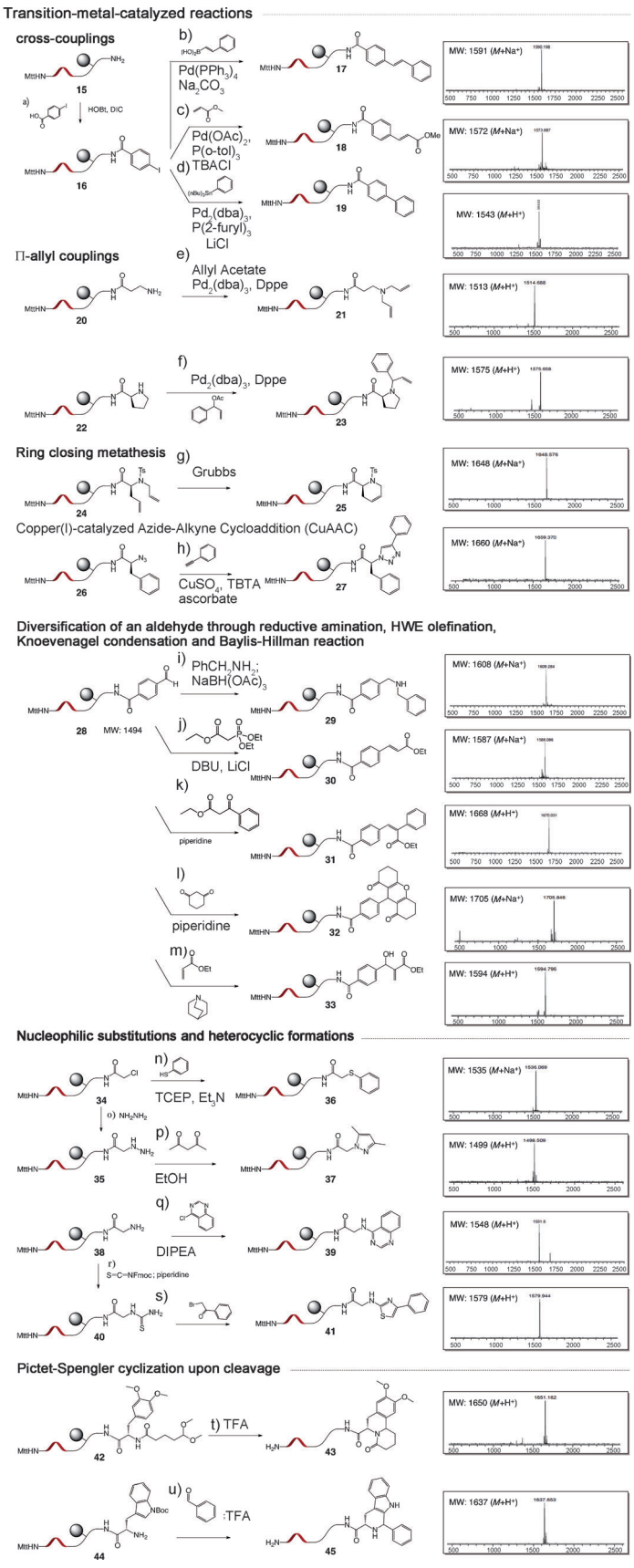


Figure 3. Left: Plot of the fluorescent intensities measured for the perfect matched hybridization (PM) and brightest mismatched hybridization (MM) for serine modified PNA (black) and unmodified PNA (gray); Right: Images of the array at 0.5 nM for the unmodified PNA (left) and ser-modified PNA (right). Unmodified PNA sequence: K-GAA CCC GGT GGA CG-K(Cy3); K-**G**A**A** C**C**C **G**G**T** G**G**A C**G**-K(Cy3). Bold letters denote serine-modified residues.

higher specificity for the perfect match versus mismatched compared with unmodified (average ratio of PM/MM for modified PNA and unmodified PNA are 3.2 and 2.1, respectively). These results are in good agreement with previous reports on the benefit of including γ-modifications originating from L-amino acids.

We next investigated the scope of the chemistry that could be achieved with PES, focusing particularly on reactions that have proven to be important for library synthesis in providing diversity and complexity.[14] To this end, a resin containing a first codon with the four different possible nucleobases (TGC**A**)[15] including a serine modified derivative (**A**) was used to evaluate different reactions (Figure 4). The outcomes of all reactions were assessed by MALDI and LC/MS analysis of the crude reaction cleavage product. Palladium-catalyzed reactions certainly stand as one of the most utilized reaction type in library synthesis. Resin **15** containing an Mtt-protected PNA tag was derivatized with 4-iodobenzoic acid to obtain **16**, which was engaged in Suzuki–Miyaura cross-coupling[16] with styrenyl boronic acid under classical coupling conditions (palladium tetrakis) to afford a complete cross-coupling reaction (**17**). Heck reaction[17] was equally productive, affording **18** in excellent yield. Stille cross-coupling[18] also proceeded as anticipated to yield the expected biaryl **19**. Palladium-catalyzed π-allylation[19] reactions were also found to be very productive. Treatment of PNA-tagged amine **20** with an excess of allyl acetate in the presence of 10% palladium-Dppe catalyst cleanly afforded the expected double allylation product **21**. Reaction of a PNA-tagged secondary amine (proline: **22**) with a more congested π-allyl system (1-phenyl allyl acetate) still afforded the desired compound **23** in good yield (more than 85% conversion, regioselectivity extrapolated from the same reaction performed in solution). Ring-closing metathesis (RCM) reactions have been used extensively in DOS, and ruthenium-based catalysts are known to be compatible with peptides.[20] Indeed, substrate **24** containing two terminal alkenes underwent smooth RCM with first generation Grubbs catalyst to yield **25**.[21] The Cu-catalyzed azide alkyne cycloaddition[22] has proven to be one of the most robust reactions, and azide substrate **26** was converted into the triazole **27** in excellent yield. Beyond transition-metal-catalyzed reactions, the aldehyde function is a versatile starting point for diversification. PNA-encoded aldehyde **28** could be smoothly engaged in reductive amination using $NaBH(OAc)_3$, Horner–Wadsworth–Emmons olefination (phosphonate, DBU, LiCl), or Knoevenagel condensation using piperidine to obtain **29**, **30** and **31**, respectively, in excellent yields. Interestingly, performing the Knoevenagel condensation with 1,3-cyclohexanedione yielded a double addition product resulting in formation of tetrahydroxantene **32**. Aldehyde **28** could also be engaged in the Baylis–Hillman reaction[23] with acrylates using quinuclidine to afford the addition product **33** in very good yield. Substitution reactions were also examined as an entry into privileged heterocyclic motifs.[24] Starting with chloroacetamide resin **34**, the chloride could be readily displaced by nucleophilic thiols, such as



thiophenol, to afford **36**. Conversely, the chloride could be displaced by hydrazine, which could then be further reacted with 1,3-diketones to afford pyrazoles such as **37**. Nucleophilic aromatic substitutions were also found to proceed smoothly. Reaction of PNA-encoded amine **38** with 4-chloroquinazoline using DIPEA as a base afforded the desired heterocycle **39** in excellent yield. The amino group could also be converted into a thiourea (**40**) under the action of Fmoc thioisocyanate followed by piperidine treatment. Thiourea **40** was then engaged in a reaction with a bromoketone to obtain the corresponding thiazole **41** in excellent yield. Finally, we investigated Pictet–Spengler cyclizations,[25] which are among the most prominent synthetic approaches to polyheterocyclic compounds. Whereas this reaction is performed under acidic conditions and would thus not be compatible with Mtt or the Rink linker, we reasoned that it could be achieved concomitantly with the final cleavage. Thus, taking 3,4-dimethoxyphenyl alanine and coupling dimethoxy valerate afforded resin **42**. Upon treatment with TFA to promote the cleavage and global deprotection, the cyclization did take place as anticipated, affording **43**. Alternatively, a suitably substituted amine on the resin (such as tryptophan) could be condensed with an aldehyde; the resulting imine was then treated with TFA to obtain the cyclization product **45** in excellent yield. Thus, Pictet–Spengler cyclizations are possible from both imine and *N*-acyliminium electrophiles in the context of PES.

We then turned our attention to the validation of this chemistry in the context of a library synthesis. Fmoc-protected fragments certainly represent one of the most diverse

Figure 4. Diversity-building reactions in PES. Reagents and conditions: a) 4-iodobenzoic acid (5.0 equiv), HOBt (5.0 equiv), DIC (15 equiv), NMP, 2 h; b) $[Pd(PPh_3)_4]$ (20%), *trans*-2-phenylvinyl boronic acid (8.0 equiv), Na_2CO_3 (9.0 equiv), DMF/H_2O (4:1), 80°C, 12 h; c) $Pd(OAc)_2$ (40%), P(*o*-tol)$_3$ (50%), TBACl (cat), methyl acrylate (30 equiv), DMF/H_2O/Et_3N (9:1:1), 80°C, 12 h; d) $[Pd_2(dba)_3]$ (20%), tri(2-furyl)-phosphane (40%), LiCl (cat), PhSn(*n*Bu)$_3$ (6.0 equiv), NMP, 80°C, 12 h; e) $[Pd_2(dba)_3]$ (10%), Dppe (20%), allyl acetate (3.0 equiv), CH_2Cl_2, 5 h; f) $[Pd_2(dba)_3]$ (10%), Dppe (20%), 1-phenylallyl acetate (3.0 equiv), CH_2Cl_2, 3 h; g) Grubbs I (50%), DCE, 50°C, 12 h; h) phehylacetylene (15 equiv), sodium ascorbate aq. (15 equiv), copper sulfate aq. (0.50 equiv), TBTA (1.0 equiv), NMP, 16 h; i) benzyl amine (15 equiv), $NaBH(OAc)_3$ (30 equiv), TMOF, 12 h; j) triethyl phosphonoacetate (4.0 equiv), DBU (5.0 equiv), LiCl (cat), DMF, 12 h; k) ethyl benzoylacetate (4.0 equiv), piperidine (5.0 equiv), DMF, 12 h; l) 1,3-cyclohexanedione (4.0 equiv), piperidine (5.0 equiv), DMF, 5 h; m) ethyl acrylate (40 equiv), quinuclidine (40 equiv), DMSO, 4 h; n) thiophenol (15 equiv), TCEP (10 mM), Et_3N (5%), DMF/H_2O (9:1), 50°C, 12 h; o) NH_2NH_2 (20 equiv), DIPEA (50 equiv), DMSO, 50°C, 12 h; p) 2,4-pentanedione (15 equiv), EtOH, 50°C, 12 h; q) chloroquinazoline (5.0 equiv), DIPEA (7.0 equiv), DMF, 50°C, 12 h; r) FmocNCS (3.0 equiv), pyridine (5.0 equiv), CH_2Cl_2, 3 h; s) 2-bromoacetophenone (0.1 M), dioxane, 2× 3 h; t) TFA; 12 h; u) benzaldehyde (10 equiv), TMOF, 12 h; TFA, 8 h. HOBt = hydroxybenzotriazole, DIC = *N*,*N*′-diisopropylcarbodiimide, NMP = *N*-methyl-2-pyrrolidone, TBACl = tetrabutylammonium chloride, Dppe = 1,2-bis(diphenylphosphino)ethane, Grubbs I = benzylidene-bis(tricyclohexylphosphane)dichlororuthenium(IV) dichloride, DCE = 1,2-dichloroethene, TBTA = tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine, TMOF = trimethyl orthoformate, DMSO = dimethyl sulfoxide, TCEP = (tris(2-carboxyethyl)phosphane), TFA = trifluoroacetic acid.

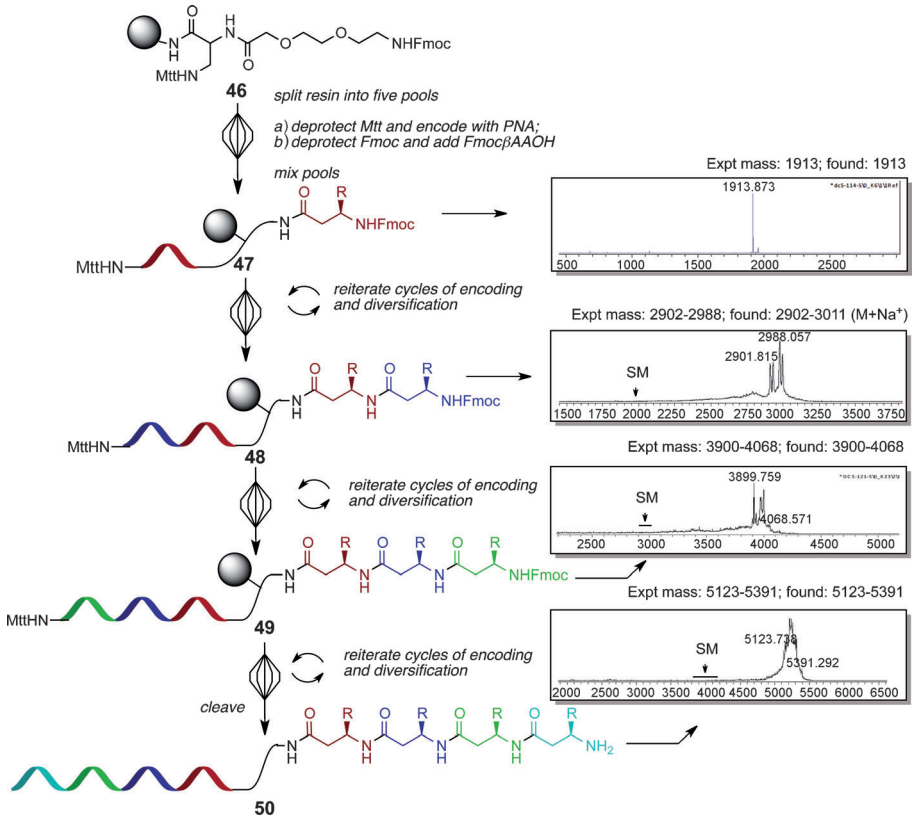


Figure 5. PES of a β-peptide library.

sources of commercially available bifunctional synthons of diversity. To illustrate the orthogonality of the Mtt-based PES with Fmoc-protected synthons, we opted for a library containing β-amino acids.[26] Such β-amino peptides are more structured and more resistant towards enzymatic degradation than their counterparts composed of α-amino acids. As shown in Figure 5, resin **46** was split into five pools and the Mtt was removed to introduce the first PNA codon (see the Supporting Information for codon sequences). Although the use of only five pools per reaction affords a total library size of 625, which does not fully capitalize on the power of PES, this restricted library was anticipated to facilitate analysis. The Fmoc was then removed and the five different Fmoc protected β-amino acids (Arg, Phe, Glu, Ile, and Ser) were coupled in their respective pool. The pools were mixed and split and the cycle of PES was reiterated three times. At each cycle, an analytical aliquot of the library was cleaved to evaluate the progression of the synthesis by MALDI MS analysis. The spectra increase in complexity because the number of compounds per pool increase from 1 in the first cycle to 5 then 25 then 125 in the second, third, and forth cycles, respectively. Nonetheless, the expected molecular weight window is clearly identified in each cycle with minimal truncation (a capping cycle is used after every coupling, hence incomplete reaction standout as lower molecular weight peaks).

Conclusion

We have developed a synthetic strategy for PNA-encoded synthesis (PES) that is broadly compatible with most reaction prototypes used in library synthesis and DOS. In addition, we have developed simple and scalable protocols for Mtt-protected PNA bearing γ-modifications. PNA-oligomers incorporating these modified residues afford higher sensitivity and fidelity in hybridization. These developments should also be applicable to parallel efforts using PNA-conjugates to organize ligands with controlled geometry.[26]

Experimental Section

Full experimental procedures and spectroscopic characterization data are provided in the Supporting Information.

Acknowledgements

This work was supported by a grant from the Institut Universitaire de France (IUF) and the European Research Council (ERC 201749). MENRT fellowships (M. C., C. Z.) as well as a fellowship from the Algerian Ministry of Higher Education and Scientific Research (DC) are also gratefully acknowledged.

[1] T. Kodadek, *Nat. Chem. Biol.* **2010**, *6*, 162–165.
[2] a) R. E. Kleiner, C. E. Dumelin, D. R. Liu, *Chem. Soc. Rev.* **2011**, *40*, 5707–5717; b) L. Mannocci, M. Leimbacher, M. Wichert, J. Scheuermann, D. Neri, *Chem. Commun.* **2011**, *47*, 12747–12753; c) M. A. Clark, *Curr. Opin. Chem. Biol.* **2010**, *14*, 396–403; d) J. L. Harris, N. Winssinger, *Chem. Eur. J.* **2005**, *11*, 6792–6801.
[3] a) G. Georghiou, R. E. Kleiner, M. Pulkoski-Gross, D. R. Liu, M. A. Seeliger, *Nat. Chem. Biol.* **2012**, *8*, 366–374; b) Z. J. Gartner, B. N. Tse, R. Grubina, J. B. Doyon, T. M. Snyder, D. R. Liu, *Science* **2004**, *305*, 1601–1605; c) S. Melkko, J. Scheuermann, C. E. Dumelin, D. Neri, *Nat. Biotechnol.* **2004**, *22*, 568–574; d) C. E. Dumelin, S. Trussel, F. Buller, E. Trachsel, F. Bootz, Y. Zhang, L. Mannocci, S. C. Beck, M. Drumea-Mirancea, M. W. Seeliger, C. Baltes, T. Muggler, F. Kranz, M. Rudin, S. Melkko, J. Scheuermann, D. Neri, *Angew. Chem.* **2008**, *120*, 3240–3245; *Angew. Chem. Int. Ed.* **2008**, *47*, 3196–3201; e) L. Mannocci, Y. Zhang, J. Scheuermann, M. Leimbacher, G. De Bellis, E. Rizzi, C. Dumelin, S. Melkko, D. Neri, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17670–17675; f) J. Scheuermann, C. E. Dumelin, S. Melkko, Y. Zhang, L. Mannocci, M. Jaggi, J. Sobek, D. Neri, *Bioconjugate Chem.* **2008**, *19*, 778–785; g) M. A. Clark, R. A. Acharya, C. C. Arico-Muendel, S. L. Belyanskaya, D. R. Benjamin, N. R. Carlson, P. A. Centrella, C. H. Chiu, S. P. Creaser, J. W. Cuozzo, C. P. Davie, Y. Ding, G. J. Franklin, K. D. Franzen, M. L.

Gefter, S. P. Hale, N. J. Hansen, D. I. Israel, J. Jiang, M. J. Kavarana, M. S. Kelley, C. S. Kollmann, F. Li, K. Lind, S. Mataruse, P. F. Medeiros, J. A. Messer, P. Myers, H. O'Keefe, M. C. Oliff, C. E. Rise, A. L. Satz, S. R. Skinner, J. L. Svendsen, L. Tang, K. van Vloten, R. W. Wagner, G. Yao, B. Zhao, B. A. Morgan, *Nat. Chem. Biol.* **2009**, *5*, 647–654; h) S. J. Wrenn, R. M. Weisinger, D. R. Halpin, P. B. Harbury, *J. Am. Chem. Soc.* **2007**, *129*, 13137–13143.

[4] a) J. P. Daguer, M. Ciobanu, S. Barluenga, N. Winssinger, *Org. Biomol. Chem.* **2012**, *10*, 1502–1505; b) J. Harris, D. E. Mason, J. Li, K. W. Burdick, B. J. Backes, T. Chen, A. Shipway, G. Van Heeke, L. Gough, A. Ghaemmaghami, F. Shakib, F. Debaene, N. Winssinger, *Chem. Biol.* **2004**, *11*, 1361–1372; c) N. Winssinger, R. Damoiseaux, D. C. Tully, B. H. Geierstanger, K. Burdick, J. L. Harris, *Chem. Biol.* **2004**, *11*, 1351–1360; d) H. D. Urbina, F. Debaene, B. Jost, C. Bole-Feysot, D. E. Mason, P. Kuzmic, J. L. Harris, N. Winssinger, *ChemBioChem* **2006**, *7*, 1790–1797; e) M. Ciobanu, K.-T. Huang, J.-P. Daguer, S. Barluenga, O. Chaloin, E. Schaeffer, C. G. Mueller, D. A. Mitchell, N. Winssinger, *Chem. Commun.* **2011**, *47*, 9321–9323; f) J. P. Daguer, M. Ciobanu, S. Alvarez, S. Barluenga, N. Winssinger, *Chem. Sci.* **2011**, *2*, 625–632; g) K. T. Huang, K. Gorska, S. Alvarez, S. Barluenga, N. Winssinger, *ChemBioChem* **2011**, *12*, 56–60; h) J. J. Diaz-Mochon, L. Bialy, M. Bradley, *Chem. Commun.* **2006**, 3984–3986; i) D. Pouchain, J. J. Diaz-Mochon, L. Bialy, M. Bradley, *ACS Chem. Biol.* **2007**, *2*, 810–818; j) N. Svensen, J. J. Diaz-Mochon, M. Bradley, *Chem. Commun.* **2011**, *47*, 7638–7640; k) N. Svensen, J. J. Diaz-Mochon, M. Bradley, *Chem. Biol.* **2011**, *18*, 1284–1289; l) N. Svensen, J. J. Diaz-Mochon, K. Dhaliwal, S. Planonth, M. Dewar, J. D. Armstrong, M. Bradley, *Angew. Chem.* **2011**, *123*, 6257–6260; *Angew. Chem. Int. Ed.* **2011**, *50*, 6133–6136.

[5] a) M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, P. E. Nielsen, *Nature* **1993**, *365*, 566–568; b) P. E. Nielsen, *Mol. Biotechnol.* **2004**, *26*, 233–248.

[6] S. Brenner, R. A. Lerner, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 5381–5383.

[7] a) X. Li, D. R. Liu, *Angew. Chem.* **2004**, *116*, 4956–4979; *Angew. Chem. Int. Ed.* **2004**, *43*, 4848–4870; b) C. T. Calderone, J. W. Puckett, Z. J. Gartner, D. R. Liu, *Angew. Chem.* **2002**, *114*, 4278–4282; *Angew. Chem. Int. Ed.* **2002**, *41*, 4104–4108; c) Z. J. Gartner, M. W. Kanan, D. R. Liu, *Angew. Chem.* **2002**, *114*, 1874–1878; *Angew. Chem. Int. Ed.* **2002**, *41*, 1796–1800; d) Z. J. Gartner, R. Grubina, C. T. Calderone, D. R. Liu, *Angew. Chem.* **2003**, *115*, 1408–1413; *Angew. Chem. Int. Ed.* **2003**, *42*, 1370–1375; e) C. T. Calderone, D. R. Liu, *Angew. Chem.* **2005**, *117*, 7549–7552; *Angew. Chem. Int. Ed.* **2005**, *44*, 7383–7386; f) B. N. Tse, T. M. Snyder, Y. Shen, D. R. Liu, *J. Am. Chem. Soc.* **2008**, *130*, 15611–15626; g) D. R. Halpin, P. B. Harbury, *PLoS Biol.* **2004**, *2*, E174; h) D. R. Halpin, P. B. Harbury, *PLoS Biol.* **2004**, *2*, E173; i) D. R. Halpin, J. A. Lee, S. J. Wrenn, P. B. Harbury, *PLoS Biol.* **2004**, *2*, 1031–1038; j) F. Buller, L. Mannocci, Y. Zhang, C. E. Dumelin, J. Scheuermann, D. Neri, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5926–5931; k) M. H. Hansen, P. Blakskjaer, L. K. Petersen, T. H. Hansen, J. W. Hoejfeldt, K. V. Gothelf, N. J. V. Hansen, *J. Am. Chem. Soc.* **2009**, *131*, 1322–1327.

[8] a) F. Debaene, J. A. Da Silva, Z. Pianowski, F. J. Duran, N. Winssinger, *Tetrahedron* **2007**, *63*, 6577–6586; b) F. Debaene, L. Mejias, J. L. Harris, N. Winssinger, *Tetrahedron* **2004**, *60*, 8677–8690; c) S. Pothukanuri, Z. Pianowski, N. Winssinger, *Eur. J. Org. Chem.* **2008**, 3141–3148.

[9] B. Sax, F. Dick, R. Tanner, J. Gosteli, *Pept. Res.* **1992**, *5*, 245–246.

[10] a) A. Aletras, K. Barlos, D. Gatos, S. Koutsogianni, P. Mamos, *Int. Enamelist Int. J. Peptide Protein. Res.* **1995**, *45*, 488–496; b) A. Isidro-Llobet, M. Alvarez, F. Albericio, *Chem. Rev.* **2009**, *109*, 2455–2504.

[11] D. Will, G. Breipohl, D. Langner, J. Knolle, E. Uhlmann, *Tetrahedron* **1995**, *51*, 12069–12082.

[12] a) B. Sahu, I. Sacui, S. Rapireddy, K. J. Zanotti, R. Bahal, B. A. Armitage, D. H. Ly, *J. Org. Chem.* **2011**, *76*, 5614–5627; b) S. Pensato, M. Saviano, N. Bianchi, M. Borgatti, E. Fabbri, R. Gambari, A. Romanelli, *Bioorg. Chem.* **2010**, *38*, 196–201; c) A. Dragulescu-Andrasi, S. Rapireddy, B. M. Frezza, C. Gayathri, R. R. Gil, D. H. Ly, *J. Am. Chem. Soc.* **2006**, *128*, 10258–10267.

[13] I. F. Pickersgill, H. Rapoport, *J. Org. Chem.* **2000**, *65*, 4048–4057.

[14] a) T. E. Nielsen, S. L. Schreiber, *Angew. Chem.* **2008**, *120*, 52–61; *Angew. Chem. Int. Ed.* **2008**, *47*, 48–56; b) S. L. Schreiber, *Science* **2000**, *287*, 1964–1969.

[15] Bold letters denote serine-modified residues.

[16] N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457–2483.

[17] J. K. Stille, *Angew. Chem.* **1986**, *98*, 504–519; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508–523.

[18] B. M. Trost, *Acc. Chem. Res.* **1980**, *13*, 385–393.

[19] H. E. Blackwell, R. H. Grubbs, *Angew. Chem.* **1998**, *110*, 3469–3472; *Angew. Chem. Int. Ed.* **1998**, *37*, 3281–3284.

[20] P. Schwab, R. H. Grubbs, J. W. Ziller, *J. Am. Chem. Soc.* **1996**, *118*, 100–110.

[21] a) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; b) C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.

[22] D. Basavaiah, A. J. Rao, T. Satyanarayana, *Chem. Rev.* **2003**, *103*, 811–891.

[23] M. E. Welsch, S. A. Snyder, B. R. Stockwell, *Curr. Opin. Chem. Biol.* **2010**, *14*, 347–361.

[24] a) B. E. Maryanoff, H. C. Zhang, J. H. Cohen, I. J. Turchi, C. A. Maryanoff, *Chem. Rev.* **2004**, *104*, 1431–1628; b) T. E. Nielsen, M. Meldal, *J. Comb. Chem.* **2005**, *7*, 599–610.

[25] a) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219–3232; b) D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, 2015–2022.

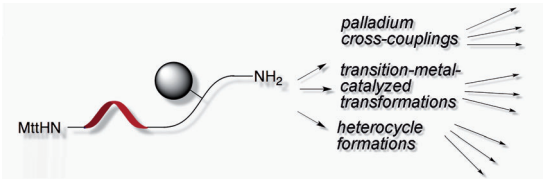
[26] a) E. A. Englund, D. Wang, H. Fujigaki, H. Sakai, C. M. Micklitsch, R. Ghirlando, G. Martin-Manso, M. L. Pendrak, D. D. Roberts, S. R. Durell, D. H. Appella, *Nat. Commun.* **2012**, 3; b) K. Gorska, K.-T. Huang, O. Chaloin, N. Winssinger, *Angew. Chem.* **2009**, *121*, 7831–7836; *Angew. Chem. Int. Ed.* **2009**, *48*, 7695–7700; c) Z. L. Pianowski, N. Winssinger, *Chem. Soc. Rev.* **2008**, *37*, 1330–1336; d) C. Scheibe, A. Bujotzek, J. Dernedde, M. Weber, O. Seitz, *Chem. Sci.* **2011**, *2*, 770–775.

Received: April 19, 2012
Revised: June 15, 2012
Published online: ■■ ■■, 2012

Combinatorial Chemistry

*D. Chouikhi, M. Ciobanu, C. Zambaldo, V. Duplan, S. Barluenga, N. Winssinger** ..... ■■■■–■■■■

Expanding the Scope of PNA-Encoded Synthesis (PES): Mtt-Protected PNA Fully Orthogonal to Fmoc Chemistry and a Broad Array of Robust Diversity-Generating Reactions



Keeping track of a library: A strategy that is compatible with a large palette of reactions that have been productively used in diversity oriented synthesis (palladium cross-couplings, metathesis, π-allylation, CuAAC, reductive amination, amidation, heterocycle formation, nucleophilic addition, conjugate additions, Pictet–Spengler cyclization) is described (see figure).