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Synthesis of a PNA-encoded cysteine protease inhibitor library☆

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Abstract—Peptide nucleic acids (PNAs) have been used to encode a combinatorial library whereby each compound is labeled with a PNA tag which reflects its synthetic history and localizes the compound upon hybridization to an oligonucleotide array. We report herein the full synthetic details for a 4000 member PNA-encoded library targeted towards cysteine protease.

1. Introduction

Microarray based technologies have proven to be a powerful analytical tool for their ability to consider large numbers of data points in a miniaturized format. Such microarrays can be prepared by several techniques including photolithography, contact printing and inkjet to generate arrays with densities ranging from 1000 to 500,000 features per square centimeters.[1] The success of microarrays in genomic analysis[2] has prompted researchers in other areas to adopt this format. To date, a number of chemistries have been developed to derivatize glass surfaces for protein,[3] oligosaccharide[4] and small molecule microarrays.[5] A strong motivation for developing small molecule microarrays is to miniaturize high throughput screening. Considering that an arrayed glass slide prepared by contact printing can present 10,000 analytes to be screened in 50 μl, this represent a 1000 fold miniaturization over the 1536 microtiter plate format. Another application of small molecule microarrays is to measure the functional activity of important enzymes[6] such as kinases or proteases from complex biological mixtures. Recently, peptide nucleic acid (PNA) tags have been used to label proteins[7] and encode small molecules[8] such that hybridization to an oligonucleotide microarray (Fig. 1) localizes the tagged entity to its preprogrammed location. An added advantage of using this strategy in combinatorial synthesis is that libraries synthesized in a split and mix format[9] can be decoded in a single operation with no redundant library members. Furthermore, the library can be screened in solution prior to hybridization which should reduce nonspecific interactions and allows the separation of bound and unbound ligand by size exclusion. This latter point is important since, by including a label with the PNA tag, the isolated ligands can be identified directly upon hybridization thereby avoiding the necessity to label the target.[8]

The library reported herein was targeted towards cysteine proteases for their well know involvement in a number of diseases[10] including tumor growth,[11] osteoporosis,[12] inflammation, neurodegenerative diseases[13] apoptosis misregulation,[14] and infectious diseases such malaria,[15] African sleeping sickness and Chaga's disease. It is important to note that in at least eight cases tested, the PNA tag and its linker did not interfere with the biological activity of the inhibitor.

2. Results and discussion

2.1. Synthetic strategy

The general structure of the PNA-encoded library **1** is

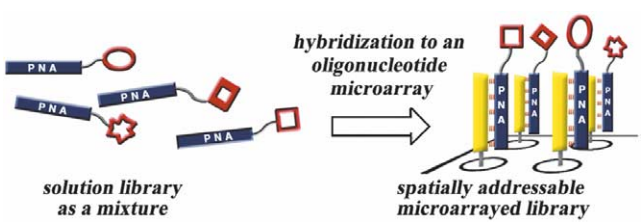

Figure 1. Hybridization of PNA-encoded library to an oligonucleotide microarray.

☆ Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.tet.2004.05.107

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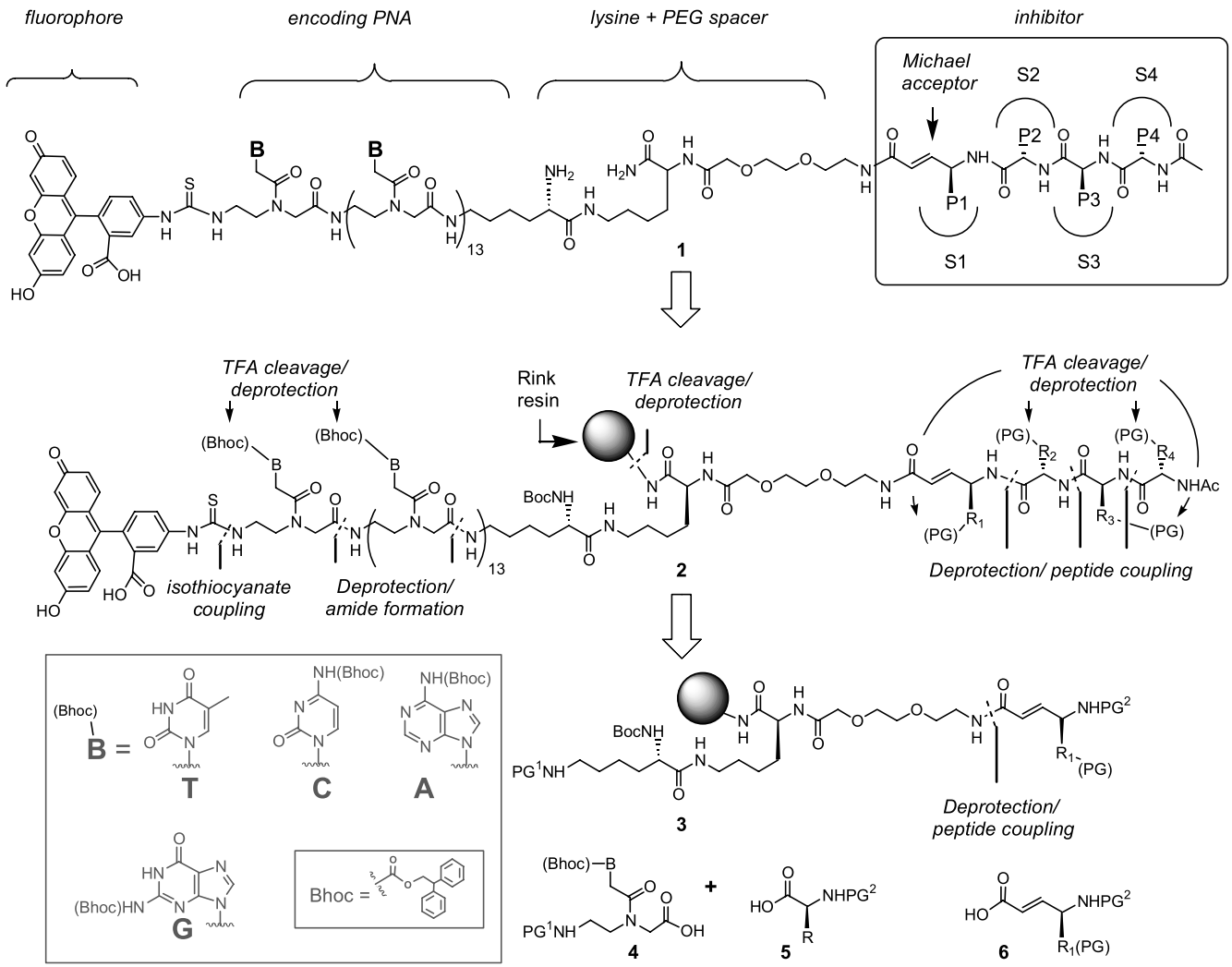
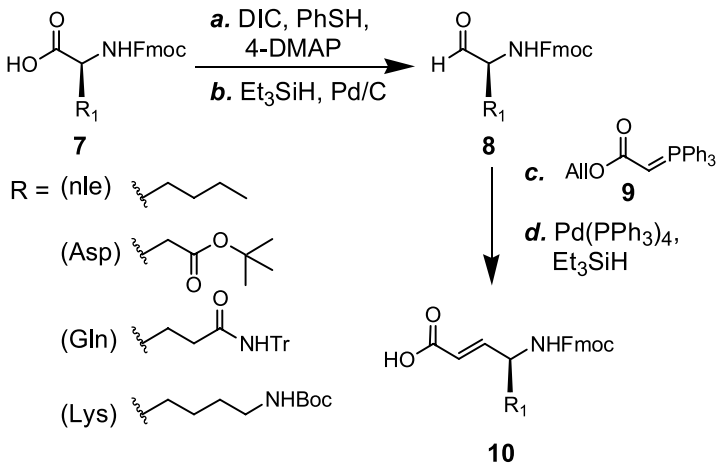


Figure 2. Design and retrosynthetic analysis of PNA-encoded library.

shown in Figure 2. The inhibitor is based on an acrylate functionality[16] that upon binding in the enzymatic pocket can be subject to a Michael addition of cysteine's nucleophilic thiol. The use of amino acids as the source of diversity was designed to maximize the potential of finding a protease–ligand interaction. A spacer (ca. 20 Å) containing a bis-ethyleneglycol moiety and a free amino group was included between the inhibitor and the PNA to insure good water solubility and reduce the risk of aggregation of the final library. Fluorescein was selected as the label based on the fact that it is excited with a green argon laser available in a number of microarray scanners, it can be potentially amplified with anti-fluorescein antibodies and it can be easily introduced from the commercially available isothiocyanate (FITC). Library **1** was anticipated to come from polymer-bound library **2** using a Rink acid labile linker, acid labile protecting groups for the PNAs' nucleobases as well as for the amino acid side chains. Two orthogonal protecting groups (PG^1 and PG^2) are required to carry out the alternative oligomerization of the PNA and inhibitor from **3** using the suitably protected monomers **4**–**6**. For this purpose, Fmoc and Alloc protecting group were selected for their mutual orthogonality and the accessibility of the corresponding monomers **4**–**6**.

2.2. Alloc-protected PNA and Fmoc-protected inhibitor oligomer (PG1=Alloc, PG2=Fmoc)

The required Fmoc protected acrylic amino acids **10** were prepared from commercially available Fmoc-protected amino acids **7** as shown in Scheme 1. Thus, esterification of the acids **7** (Nle, Asp, Gln, Lys) with ethane thiol afforded the corresponding thioesters which were reduced to aldehydes **8** with palladium on charcoal and triethyl silane in good yield.[17] The aldehydes **8** were then condensed with commercially available ylide **9** to afford the allyl acrylic esters which were deprotected with palladium-catalyzed allyl transfer to yield the desired Fmoc protected acrylic amino acids **10**. The required Alloc-protected PNA monomers **14** were most conveniently obtained by exchanging the Fmoc protecting group from commercially available PNA monomers **11** while temporarily loading them on a 2-chlorotrityl resin (Scheme 2) thereby avoiding all purifications and workups. Thus, acids **11** were coupled to 2-chlorotrityl resin to obtain polymer-bound esters **12**. Fmoc deprotection with DBU[18] and reprotection with allyl chloroformate yielded the Alloc protected esters **13** which were released from the resin using acetic acid in trifluoroethanol/dichloromethane[19] to obtained suitably

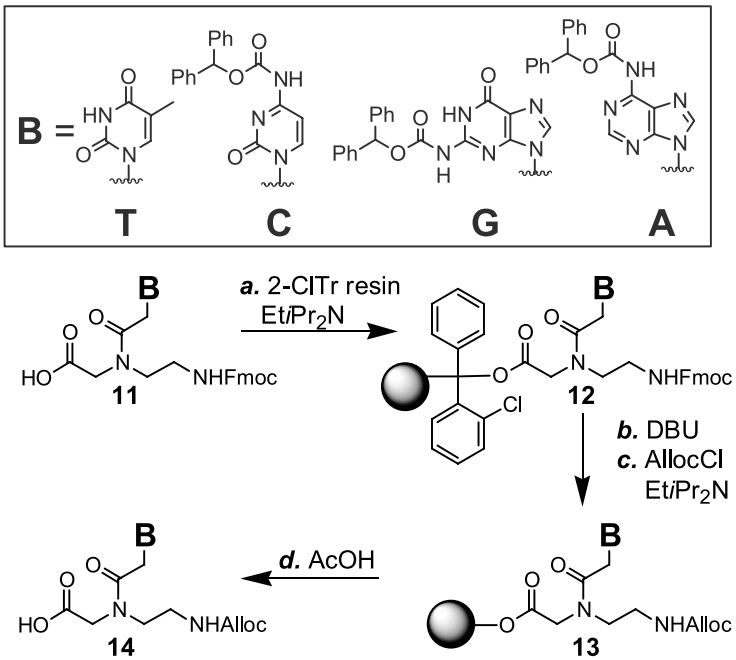


Scheme 1. Synthesis of Fmoc protected acrylic amino acids **10**. Reagents and conditions: (a) **7** (1.0 equiv.), DIC (1.6 equiv.), PhSH (1.1 equiv.), 4-DMP (0.05 equiv.), CH_2Cl_2, 23 °C, 30 min, 94% for Asp, 88% for nle, 95% for Gln, 94% for Lys; (b) Et_3SiH (4.0 equiv.), 5% Pd/C (0.02 equiv.), CH_2Cl_2, 23 °C, 90 min, 77% for **8**-**Nle**, 87% for **8**-**Asp**, 94% for **8**-**Gln**, 80% for **8**-**Lys**; (c) **9** (1.6 equiv.), Et*i*Pr_2N (1.4 equiv.), toluene, 80 °C, 90 min, 86% for Nle, 83% for Asp, 90% for Gln, 834% for Lys; (d) Bu_3SnH (3.0 equiv.), $Pd(PPh_3)_4$ (0.1 equiv.), CH_2Cl_2, 23 °C, 90 min, 86% for **10**-**Nle**, 82% for **10**-**Asp**, 89% for **10**-**Gln**, 91% for **10**-**Lys**.

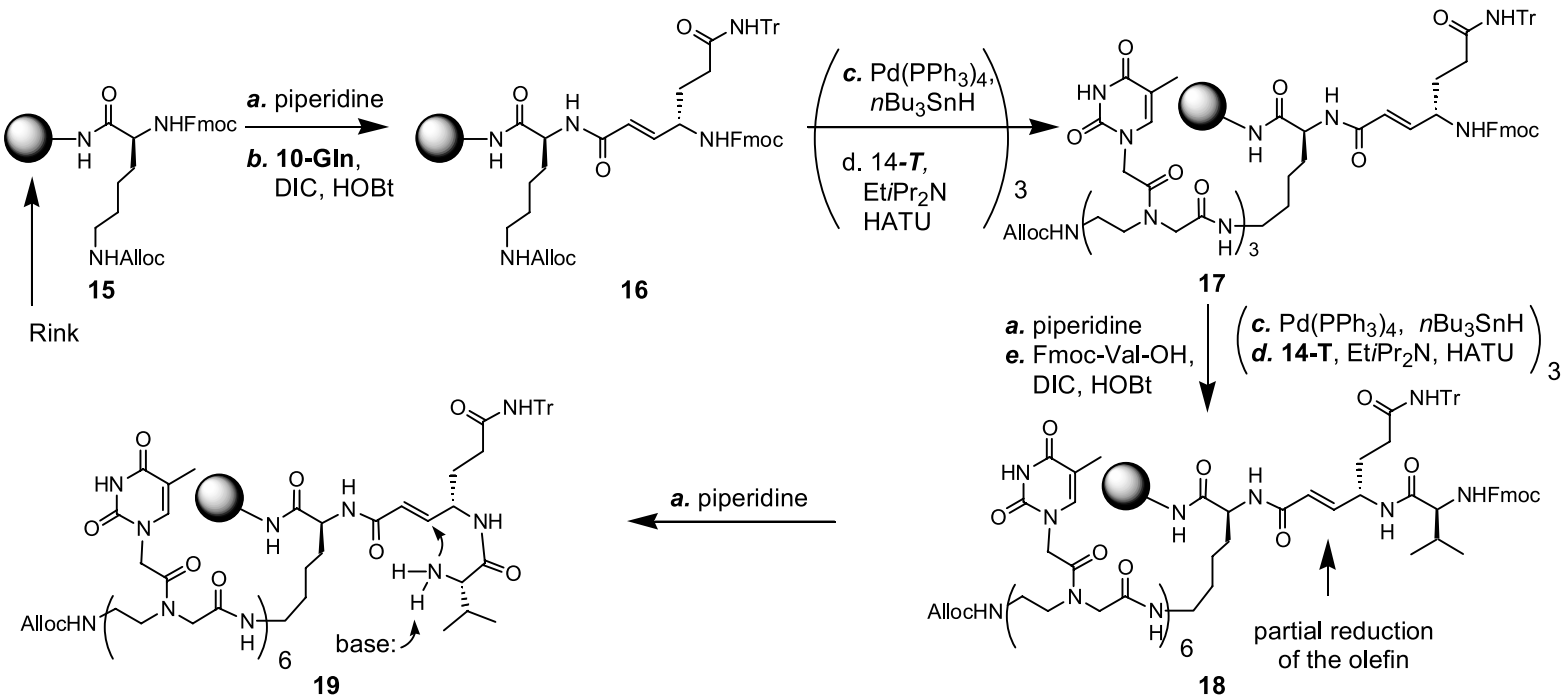
protected PNA monomer **14**. TFA cleavages with as little as 1% TFA in CH_2Cl_2 lead to partial Bhoc deprotection in 5 min. With the required building blocks at hand we turned our attention to the co-synthesis of the PNA and inhibitor. As shown in Scheme 3, Rink resin loaded with the orthogonally protected *N*-α-Fmoc-*N*-ε-Alloc-lysine (**15**) was substitute with the acrylic amino acid **10**-**Gln** to obtain resin **16**. The Alloc group was removed and a representative codon was introduced by reiteratively coupling PNA monomer **14**-**T** to obtain compound **17**. Sequential Fmoc deprotection, introduction of a second amino acid, Alloc deprotection and introduction of a second codon afforded compound **18** which was engaged in a third cycle. During this synthesis, two side reactions that could potentially compromise the purity of a final library were observed. First, during certain Alloc deprotection [$(Pd(PPh_3)_4$, *n*Bu_3SnH], partial reduction of the acrylate olefin was observed and second, basic conditions used in the Fmoc deprotection (piperidine or DBU) for compound **18** lead to partial piperazinone formation via intermediate **19**. With these results, we reasoned that it would be prudent to reverse the protecting group strategy since the Alloc deprotections may be carried out under acidic condition and would avoid the piperazinone formation and, the reduced number of Alloc deprotections should minimize the level of acrylate reduction.

2.3. Fmoc-protected PNA and Alloc-protected inhibitor oligomer (PG1=Fmoc, PG2=Alloc)

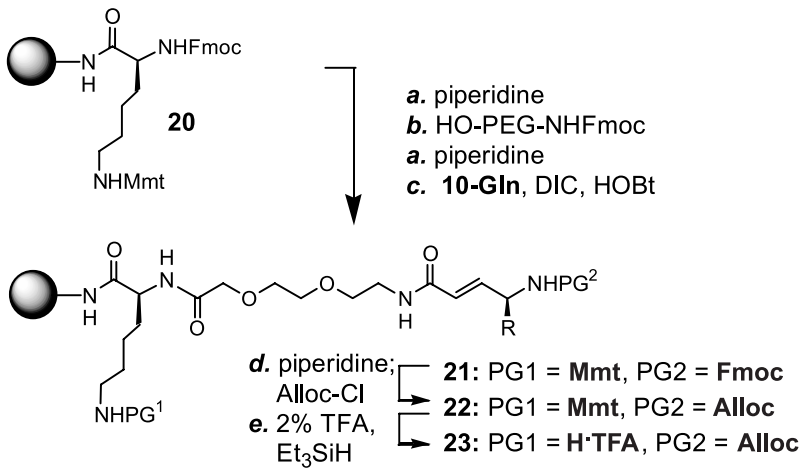
Rink resin loaded with the orthogonally protected



Scheme 2. Preparation of Alloc-protected PNA monomers **14**. Reagents and conditions: (a) **11** (1.0 equiv.), Et*i*Pr_2N (1.0 equiv.), resin (2.0 equiv.), CH_2Cl_2, 23 °C, 3 h; (b) DBU (1.0 equiv.) CH_2Cl_2, 23 °C, 5 min; (c) Alloc-Cl (3.0 equiv.), Et*i*Pr_2N (4.0 equiv.), CH_2Cl_2, 0 °C, 2×10 min; (d) AcOH–CF_3CH_2OH–CH_2Cl_2 (1:1:8), 60 min, precipitation in Et_2O, 60% for **14**-**T**, 46% for **14**-**C**, 61% for **14**-**G**, 58% for **14**-**A**, average purity >95%.

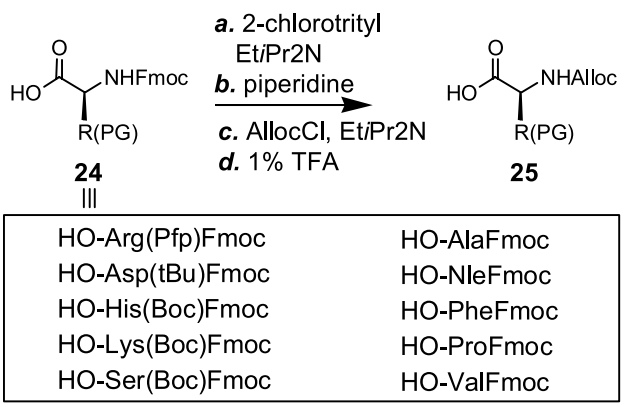


Scheme 3. Optimization of conditions for the co-synthesis of PNA and peptides using Alloc-protected PNA and Fmoc-protected amino acids. Reagents and conditions: (a) 20% piperidine, DMF, 23 °C, 2.5 min; (b) **10-Gln** (2.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF, 23 °C, 3 h; (c) nBu$_3$SnH (10 equiv.), Pd(PPh$_3$)$_4$ (0.2 equiv.), CH_2Cl_2, 23 °C, 30 min; (d) **14-T** (4.0 equiv.), EtiPr$_2$N (4.0 equiv.), HATU (3.5 equiv.), DMF, 23 °C, 1 h.



Scheme 4. Preparation of Alloc-protected acrylamide **23**. Reagents and conditions: (a) 20% piperidine, DMF, 23 °C, 2.5 min; H·TFA/Alloc (2.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF, 23 °C, 6 h; (c) **10-Gln** (2.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF, 23 °C, 6 h; (d) 20% piperidine, DMF, 23 °C, 2.5 min; Alloc-Cl (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), DMF, 0 °C, 30 min; (d) 1% TFA, 5% Et$_3$SiH, CH_2Cl_2, 23 °C, 1 h.

N-α-Fmoc-*N*-ε-4-methyltrityl-lysine (**20**, Scheme 4) was substitute with the ethyleneglycol spacer followed by acrylic amino acid **10-Gln** to obtain resin **21**. The Fmoc group was exchanged for an Alloc and the methyltrityl was removed to obtain compound **23** as a TFA salt via **22**. The required Alloc-protected amino acids **25** with the appropriate side chain protecting groups could be readily obtained from the corresponding Fmoc protected amino acids **24** (Scheme 5) by temporarily loading them on a chlorotrityl resin. As for the PNA, this procedure avoided all work ups and purifications while delivering the amino acids with the appropriate side chain protecting groups. We then preceded to the synthesis of a set of representative PNA encoded inhibitors of which an example is Scheme 6. Thus, alternative addition of a PNA codon by reiterative Fmoc deprotection/coupling followed by Alloc deprotection and amino acid coupling led after labeling with FITC and cleavage from the resin to compound **26**. The use of triethyl silane[20] rather than tributyl tin hydride for the Alloc deprotection, while slower, proved to be more reliable and avoided all acrylate reduction problems. The final compound **26** was thus obtained from intermediate **20** in 40 steps. MALDI analysis of the final product shows the desired compound as a single major compound (Fig. 3). The relatively small number of truncated sequences reflects the efficiency of the well-established coupling procedures[21] and of the protecting group strategy.



Scheme 5. Exchange of protection groups for amino acid monomers. Reagents and conditions: (a) **24** (1.4 equiv.), Et*i*Pr$_2$N (2.0 equiv.), resin (1.0 equiv.) CH_2Cl_2, 23 °C, 3 h; (b) 20% piperidine in DMF, 23 °C, 10 min; (c) Alloc-Cl (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), CH_2Cl_2, 0 °C, 30 min; (d) 2.5% TFA in CH_2Cl_2, 23 °C, 30 min, 78% average yield, >95% purity by NMR.

2.4. Split and mix synthesis of the library

The codon system shown in Figure 4 was designed based on the following criteria: At least 2 base pair mismatches between each codon, no more than 6 consecutive purines and homogeneous hybridization properties with 4-letter codons at the extremities. The synthesis of the library was executed as shown in Scheme 7. The completion of each reaction was verified by mass spectroscopy analysis, a spectra of a representative pool is shown in Figure 5 (remaining spectra's are shown in the Supplementary Material). Thus, the four library pools (**23**) were encoded with their respective PNA sequences to obtain four pools of **27** which were mixed and subjected to a common Alloc deprotection before being split into ten new pools. To each

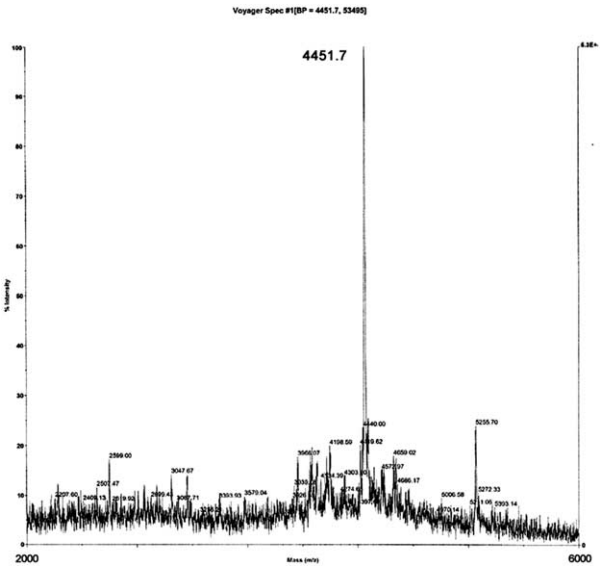
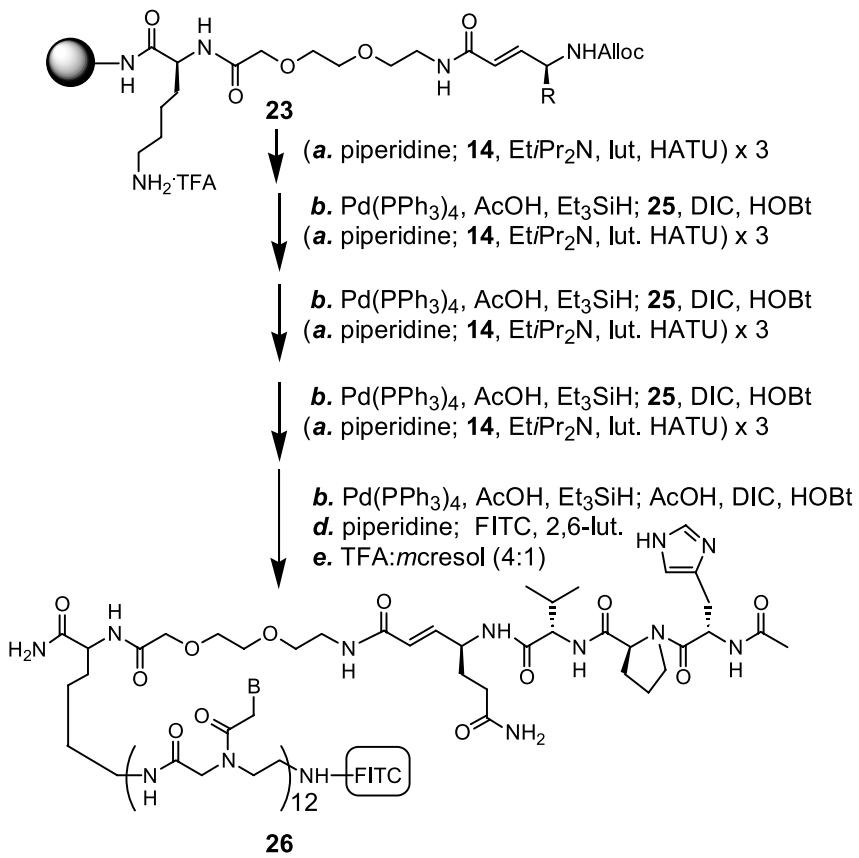


Figure 3. MALDI analysis of compound **26**.



Scheme 6. Optimized synthesis of PNA-encode inhibitor **26**. (a) 20% piperidine, DMF, 23 °C, 2.5 min; (b) **14**, Et*i*Pr$_2$N (4.0 equiv.), 2,6-lut. (6.0 equiv.), HATU (3.5 equiv.), DMF, 23 °C, 1 h; (c) Et_3SiH (10 equiv.), $Pd(PPh_3)_4$ (0.2 equiv.), AcOH (10 equiv.), DMF/CH_2Cl_2 (1/1), 23 °C, 30 min; **25** (4.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF , 23 °C, 2 h; (d) 20% piperidine, DMF, 23 °C, 2.5 min; FITC (10 equiv.), lut. (10 equiv.), DMF, 23 °C 5 h; (e) TFA: *m*cresol (4:1), 23 °C, 2 h, Et_2O precipitation, centrifugation.

pool was added the second element of diversity followed by its respective PNA codon to obtain ten pools of **28** each containing 4 compounds. The pools were mixed deprotected and re-split into 10 new pools which were subjected to another round of diversification/encoding to yield ten pools of **29** each containing 40 compounds. While the identity of every component of a pool could no longer be identified by mass spectroscopy, the absence of lower molecular weight peaks suggests that all the steps thus far were very high yielding as was observed in a number of test cases such as compound **26**. The ten pools of **29** were once more mixed, deprotected, split and the last amino acid followed by the last codon was introduced to obtain 10 pools of **30** which

R^1	R^2	R^3	R^4
	Ala = TCC	CAA	CAAC
Asp = CCCA	Nle = CTC	ACA	ACAC
Gln = GGGT	Val = CCT	AAC	AACC
Nle = GGCA	Pro = TGG	CTT	CTTC
Lys = CCGT	Phe = GTG	TCT	TCTC
	His = GGT	TTC	TTCC
	Arg = AGC	ATG	ATGC
	Lys = GAC	TAG	TAGC
	Ser =CGA	GTA	GTAC
	Asp = CAG	AGT	AGTC

Figure 4. Sequence of the assigned codons.

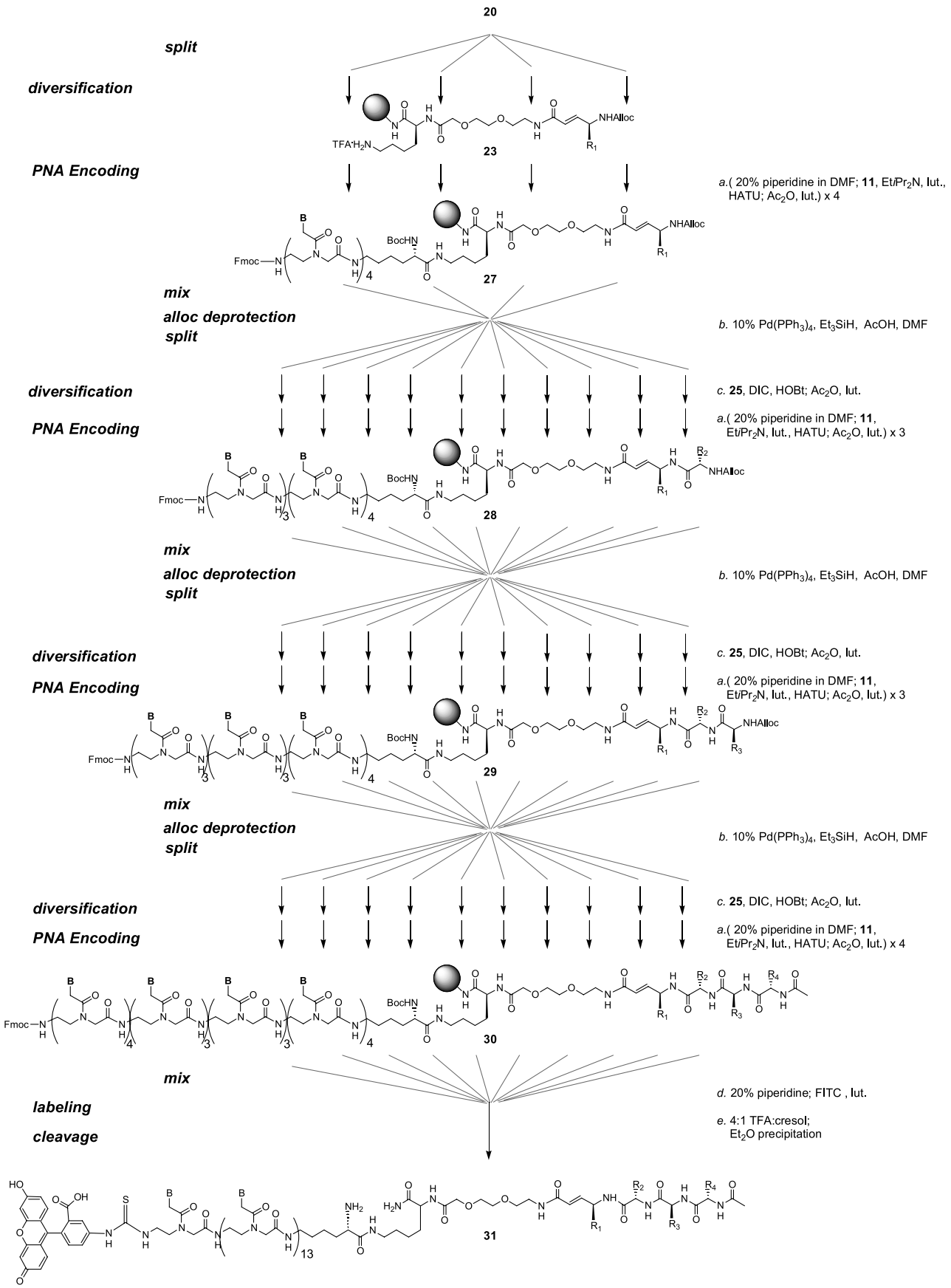
were combined. MALDI analysis was no longer useful to evaluate this last step as there are too many compounds in the mixture. The last Fmoc was removed and the library was labeled with fluorescein isothiocyanate (FITC). Finally, the library was cleaved as a mixture of 4000 compounds with TFA/cresol and precipitated in diethyl ether to obtain the library free of protecting group byproducts. Quantification of the fluorescein indicates a 32% recovery based on the loading of starting resin **20**.

A PNA-encoded library of 4000 compounds by split and mix synthesis was achieved. The use of this library to profile protease activity and identify inhibitors for subsequent biological chemistry study will be reported shortly. While the Alloc-protected PNA monomers proved to be less suitable than the corresponding Fmoc monomers for the present library, their chemistry should be applicable to other libraries and, by virtue of their stability to basic condition, further extend the scope of reactions to bring about molecular diversity with PNA encoding.

3. Experimental

3.1. General techniques

All reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous



Scheme 7. PNA-encode split and mix synthesis of library **31**. Reagents and conditions: (a) 20% piperidine, DMF, 23 °C, 2.5 min; **14** (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), HATU (3.5 equiv.), 2,6-lut. (6.0 equiv.), DMF, 23 °C, 1 h; Ac_2O (4.0 equiv.), lut. (4.0 equiv.), DMF, 23 °C, 5 min; (b) Et_3SiH (10 equiv.), $Pd(PPh_3)_4$ (0.2 equiv.), AcOH (10 equiv.), DMF/CH_2Cl_2 (1/1), 23 °C, 30 min; (c) **25** (4.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.), DMF, 23 °C, 2 h; Ac_2O (4.0 equiv.), lut. (4.0 equiv.), DMF, 23 °C, 5 min; (d) 20% piperidine, DMF, 23 °C, 2.5 min; FITC (10 equiv.), lut. (10 equiv.), DMF, 23 °C, 5 h; (e) TFA–cresol (4:1), 23 °C, 3 h; precipitated in Et_2O.

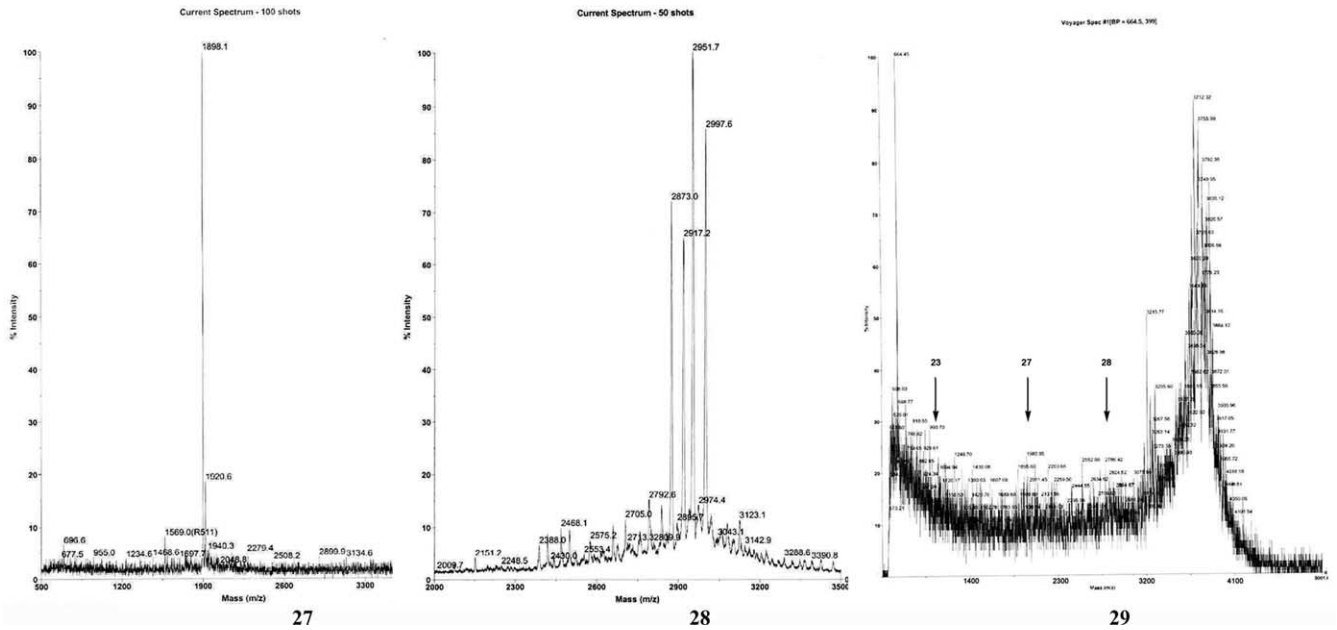


Figure 5. MALDI analysis of representative pools along the library synthesis.

conditions, unless otherwise noted. All solid phase reactions were carried out at ambient temperature unless specified otherwise. Tetrahydrofuran (THF), toluene and diethyl ether (Et_2O) were distilled from sodium–benzophenone, and methylene chloride (CH_2Cl_2) from calcium hydride. Anhydrous solvents were also obtained by passing them through commercially available activated alumina columns (Solv-Tek, Inc., VA). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Substituted polystyrene resins (100–200 mesh, 1% DVB) and amino acids were purchased from Advanced Chemtech or Novabiochem. PNA monomers and polyethyleneglycol spacer were purchased from Applied Biosystems. Solution reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light for visualization agent and 10% ethanolic phosphomolybdic acid, ninhydrin or vanillin solution and heat, as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Bruker Advance-400 instruments and calibrated using residual undeuterated solvent as an internal reference. Multiplicities were abbreviated as: s=singlet, d=doublet, t=triplet, q=quartet, qt=quintet, m=multiplet, b=broad. IR spectra were recorded on a Perkin–Elmer 1600 series FT-IR spectrometer. HRMS were recorded on a Bruker micro-TOF instrument (ESI) while MALDIs were performed on an Applied Biosystem Voyager.

3.2. Fmoc-protected acrylic amino acids 10

General procedure for the thioesterification. To a stirring solution of Fmoc protected amino acid **7** in CH_2Cl_2 (0.2–0.3 M) was added thioethanol (1.2 equiv.), followed by *N*,*N*-diisopropylcarbodiimide (1.6 equiv.) and 4-DMAP (0.05 equiv.). The reaction mixture was stirring for 25–30 min at 23 °C then loaded directly onto a silica gel column and eluted with EtOAc–hexanes.

3.2.1. Ethyl *N*-Fmoc-*O*-*t*Butyl-thioaspartate. 94% yield as viscous oil; R_f=0.75 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3339.9, 2976.3, 1731.1, 1503.4, 1450.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.78 (2H, d, J=7.0 Hz, ArH), 7.68 (2H, m, ArH), 7.45 (2H, m, ArH), 7.34 (2H, m, ArH), 6.15 (1H, m, NH), 4.73 (1H, m, CH), 4.60 (1H, m, Fmoc–CH_2), 4.39 (1H, m, Fmoc–CH_2), 4.30 (1H, t, J=7.0 Hz, Fmoc–CH), 3.05 (1H, dd, J=17.0, 4.5 Hz, CH_2CO), 2.92 (2H, q, J=7.5 Hz, SCH_2CH$_3$), 2.75 (1H, dd, J=17.0, 4.5 Hz, CH_2CO), 1.49 (9H, s, *t*Bu), 1.29 (3H, t, J=7.0 Hz, SCH$_2$$CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 200.3, 170.1, 155.94, 143.9, 143.7, 141.3, 127.8, 127.1, 125.2, 125.1, 120.0, 82.0, 67.4, 57.4, 47.2, 37.5, 28.0, 23.6, 23.6, 14.4; HRMS (ESI) $C_{25}H_{29}NNaO_5S$ calcd for $(MNa)^+$: 478.1661, found: 478.1636.

3.2.2. Ethyl *N*-Fmoc-thionorleucinate. 88% yield as white solid; R_f=0.78 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3305.5, 2951.9, 1694.1, 1535.6, 1450.4 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (2H, m, ArH), 7.52 (2H, m, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.26 (1H, d, J=9.0 Hz, NH), 4.53 (1H, m, CH), 4.42 (2H, m, Fmoc–CH_2), 4.28 (1H, t, J=7.0 Hz, Fmoc–CH), 2.92 (2H, q, J=8.0 Hz, SCH_2CH$_3$), 1.92 (1H, m, CH_2CH$_2$CH$_2$CH$_3$), 1.65 (1H, m, CH_2CH$_2$CH$_2$CH$_3$), 1.37 (4H, m, CH$_2$$CH_2$$CH_2CH_3$), 1.29 (3H, t, J=8.0 Hz, SCH$_2$$CH_3$), 0.94 (3H, m, CH$_2CH_2$-CH$_2$$CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 201.0, 155.8, 143.9, 143.7, 141.3, 127.7, 127.1, 125.1, 125.0, 120.0, 67.0, 61.0, 47.2, 32.6, 27.3, 23.28, 22.2, 14.5, 13.8; HRMS (ESI) calcd for $C_{23}H_{27}NNaO_3S$ $(MNa)^+$: 420.1606, found: 420.1607.

3.2.3. Ethyl *N*-Fmoc-*N*-γ-trityl-thioglutaminate. 95% yield as white solid; R_f=0.55 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3310.5, 3056.5, 2928.3, 1679.1, 1492.4, 1447.2 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (2H, d, J=7.5 Hz, ArH), 7.64 (2H, m, ArH), 7.42 (2H, m, ArH), 7.32 (2H, m, ArH), 6.85 (1H, s, NH), 7.25 (2H, m, Ar–H), 5.88 (1H, d, J=7.0 Hz, NH), 4.56 (1H, m, CH), 4.37 (2H, m,

Fmoc–CH_2), 4.27 (1H, t, J=7.0 Hz, Fmoc–CH), 2.91 (2H, q, J=7.0 Hz, S$CH_2$$CH_3$), 2.42 (2H, m, $CH_2$$CH_2$CO), 2.23 (1H, m, $CH_2$$CH_2$CO), 2.04 (1H, m, $CH_2$$CH_2$CO), 1.27 (3H, t, J=7.0 Hz, SCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 201.0, 171.0, 156.2, 144.5, 143.9, 143.7, 141.3, 128.7, 128.0, 127.8, 127.7, 125.3, 125.2, 120.0, 70.7, 67.1, 47.3, 33.3, 27.9, 23.3, 14.4; HRMS (ESI) calcd for $C_{41}H_{38}N_2NaO_4S$ $(MNa)^+$: 677.2448, found: 677.2483.

3.2.4. Ethyl *N*-Fmoc-*N*-ε-Boc-thiolysinate. 94% yield as white solid; R_f=0.51 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3352.2, 2930.8, 1688.3, 1526.2, 1449.1 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (2H, d, J=8.0 Hz, ArH), 7.66 (2H, m, ArH), 7.43 (2H, m, ArH), 7.34 (2H, m, ArH), 5.52 (1H, m, NH), 4.62 (1H, s, NH), 4.55 (1H, m, CH), 4.41 (2H, m, Fmoc–CH_2), 4.27 (1H, m, Fmoc–CH), 3.14 (2H, m, $CH_2CH_2CH_2$$CH_2$NH), 2.92 (2H, q, J=7.5 Hz, S$CH_2$$CH_3$), 1.92 (1H, m, $CH_2$$CH_2CH_2CH_2$NH), 1.70 (1H, m, $CH_2$$CH_2$-$CH_2CH_2$NH), 1.47 (13H, m, *t*Bu and $CH_2$$CH_2$$CH_2CH_2$NH), 1.28 (3H, t, J=7.5 Hz, SCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 201.00, 156.2, 156.0, 143.9, 143.7, 141.3, 127.7, 127.1, 125.1, 120.0, 79.22, 67.1, 60.8, 47.2, 39.8, 32.2, 29.7, 28.4, 23.3, 22.3, 14.5; HRMS (ESI) $C_{23}H_{27}NNaO_3S$ calcd for $(MNa)^+$: 535.2240, found: 535.2222.

3.2.5. Aldehyde 8; general procedure for reduction of thioester. To a stirring solution of the thioester (vide supra) (0.2–0.3 M) and of Pd/C 5% (2 mol%) in CH_2Cl_2 was added Et_3SiH (4.0 equiv.). The reaction mixture was stirred at 23 °C until TLC analysis revealed complete consumption of the starting material (30–40 min). The crude reaction mixture was loaded directly onto a silica gel column and eluted with EtOAc–hexanes.

*N-Fmoc-O-tButyl-aspartal (**8-Asp**)*. 87% yield as viscous oil; R_f=0.56 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3355.9, 2977.8, 1724.4, 1515.7, 1450.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.67 (1H, s, HCO), 7.68 (2H, d, J=7.5 Hz, ArH), 7.63 (2H, d, J=7.5 Hz, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.99 (1H, d, J=7.0 Hz, NH), 4.46 (3H, m, Fmoc–CH_2 and CH), 4.30 (1H, t, J=7.0 Hz, Fmoc–CH), 2.97 (1H, dd, J=17.0, 4.5 Hz, CH_2CO), 2.83 (1H, dd, J=17.0, 4.5 Hz, CH_2CO), 1.49 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.8, 170.2, 156.2, 143.8, 143.7, 141.3, 127.8, 127.1, 125.1, 120.0, 82.2, 67.3, 56.6, 47.1, 35.6, 28.0; HRMS (ESI) $C_{23}H_{25}NNaO_5$ calcd for $(MNa)^+$: 418.1625, found: 418.1576.

N-Fmoc-norleucinal (**8-Nle**). 77% yield as white solid; R_f=0.73 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3321.7, 2955.0, 1738.3, 1689.0, 1537.0, 1448.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.62 (1H, s, HCO), 7.80 (2H, m, ArH), 7.64 (2H, m, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.34 (1H, d, J=9.0 Hz, NH), 4.47 (2H, d, J=7.0 Hz, Fmoc–CH_2), 4.35 (1H, m, CH), 4.26 (1H, t, J=7.0 Hz, Fmoc–CH), 1.95 (1H, m, $CH_2CH_2CH_2CH_3$), 1.66 (1H, m, $CH_2CH_2CH_2CH_3$), 1.38 (4H, m, $CH_2$$CH_2$$CH_2CH_3$), 0.95 (3H, m, $CH_2CH_2CH_2$$CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 199.3, 143.73, 141.3, 127.8, 127.1, 125.0, 120.0, 66.9, 60.2, 47.2, 28.9, 27.1, 22.5, 13.8; HRMS (ESI) calcd for $C_{21}H_{23}NNaO_3$ $(MNa)^+$: 360.1570, found: 360.1592.

N-α-Fmoc-N-γ-trityl-glutaminal (**8-Gln**). 94% yield as white solid; R_f=0.35 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3316.9, 3057.4, 1714.0, 1671.8, 1492.5, 1447.6 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.47 (1H, s, HCO), 7.70 (2H, m, ArH), 7.62 (2H, m, ArH), 7.40 (2H, m, ArH), 7.30 (15H, m, ArH), 7.23 (2H, m, ArH), 6.83 (1H, s, NH), 5.61 (1H, d, J=6.0 Hz, NH), 4.49 (1H, m, CH), 4.16 (2H, q, J=7.0 Hz, Fmoc–CH_2), 4.24 (1H, t, J=7.0 Hz, Fmoc–CH), 2.35 (2H, m, $CH_2$$CH_2$CO), 2.26 (1H, m, CH_2CH_2CO), 1.86 (1H, m, CH_2CH_2CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.6, 170.9, 156.4, 144.5, 143.7, 141.4, 128.6, 128.0, 127.7, 127.1, 125.0, 120.0, 70.7, 66.7, 59.4, 47.3, 32.5, 24.6; HRMS (ESI) calcd for $C_{39}H_{34}N_2NaO_4$ $(MNa)^+$: 617.2411, found: 617.2428.

N-α-Fmoc-N-ε-Boc-lysinal (**8-Lys**). 80% yield as white solid; R_f=0.40 (SiO_2, EtOAc–hexanes 1:1); IR (KBr): 3352.3, 2929.8, 1686.2, 1525.0, 1449.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.60 (1H, s, HCO), 7.79 (2H, d, J=7.5 Hz, ArH), 7.64 (2H, d, J=7.5 Hz, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.54 (1H, s, NH), 4.62 (1H, s, NH), 4.46 (2H, m, Fmoc–CH_2), 4.31 (1H, m, CH), 4.25 (1H, t, J=7.0 Hz, Fmoc–CH), 3.15 (2H, m, $CH_2CH_2CH_2$$CH_2$NH), 1.96 (1H, m, $CH_2$$CH_2CH_2CH_2$NH), 1.70 (1H, m, $CH_2CH_2CH_2CH_2$NH), 1.46 (13H, m, *t*Bu and $CH_2$$CH_2$$CH_2CH_2$NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 199.4, 156.2, 143.7, 141.3, 127.1, 125.0, 120.0, 79.3, 67.0, 60.0, 47.2, 40.0, 29.8, 28.5, 28.4, 22.1; HRMS (ESI) $C_{26}H_{32}N_2NaO_5$ calcd for $(MNa)^+$: 475.2204, found: 475.2210.

3.2.6. Allyl acrylic ester derivatives; general procedure for the Wittig olefination. To a stirring solution of the aldehyde **8** (0.1 M) and allylphosphonium iodide (1.6 equiv.) in toluene at 80 °C was added Et*i*Pr_2N (1.4 equiv.). The reaction mixture was stirred at 80 °C until TLC analysis revealed complete consumption of the starting material (90–120 min). The reaction mixture was diluted in EtOAc and washed with 0.1 N HCl, sat. $NaCO_3$, brine, dried over $MgSO_4$ and purified by silica gel chromatography (EtOAc–hexanes).

Allyl N-Fmoc-O-tButyl-acrylicaspartate. 83% yield as viscous oil; R_f=0.86 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3336.7, 2978.5, 1725.1, 1526.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (2H, d, J=7.5 Hz, ArH), 7.62 (2H, d, J=7.5 Hz, ArH), 7.43 (2H, m, ArH), 7.34 (2H, m, ArH), 6.97 (1H, dd, J=16.0, 5.0 Hz, CH=CHCO), 5.98 (2H, m, CH_2=$CH$$CH_2$O and CH=$CH$CO), 5.70 (1H, d, J=8.5 Hz, NH), 5.38 (1H, dd, J=17.0, 1.5 Hz, CH_2=CHCH_2-O), 5.28 (1H, dd, J=10.5, 1.5 Hz, CH_2=$CHCH_2$O), 4.75 (1H, m, CH), 4.68 (2H, d, J=6.0 Hz, CH_2=CHCH_2O), 4.45 (2H, m, Fmoc–CH_2), 4.25 (1H, t, J=7.0 Hz, Fmoc–CH), 2.68 (1H, dd, J=16.0, 5.0 Hz, CH_2CO), 2.59 (1H, dd, J=16.0, 5.0 Hz, CH_2CO), 1.47 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.8, 165.5, 155.5, 146.6, 143.8, 141.3, 129.4, 128.0, 127.5, 125.6, 120.5, 118.4, 82.0, 67.0, 65.2, 48.8, 47.2, 39.4, 28.0; HRMS (ESI) $C_{28}H_{31}NNaO_6$ calcd for $(MNa)^+$: 500.2044, found: 500.2040.

Allyl N-Fmoc-acrylicnorleucinate. 86% yield as white solid; R_f=0.73 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3303.3, 2954.2, 1727.8, 1689.7, 1540.6, 1449.8 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (2H, d, J=7.5 Hz, ArH), 7.63 (2H, d, J=7.5 Hz, ArH), 7.43 (2H, m, ArH), 7.35 (2H,

m, ArH), 6.91 (1H, dd, *J*=15.5, 5.2 Hz, *CH*═CHCO), 5.98 (2H, m, CH_2═*CH*CH_2O and CH═*CH*CO), 5.38 (1H, dd, *J*=17.0, 1.5 Hz, *CH*$_2$═CHCH_2O), 5.29 (1H, dd, *J*=10.5, 1.5 Hz, *CH*$_2$═CHCH_2O), 4.85 (1H, d, *J*=8.0 Hz, NH), 4.68 (2H, d, *J*=6.0 Hz, CH_2═CH*CH*$_2$O), 4.48 (2H, d, *J*=7.0 Hz, Fmoc–CH_2), 4.37 (1H, m, CH), 4.25 (1H, t, *J*=7.0 Hz, Fmoc–CH), 1.63 (1H, m, *CH*$_2$$CH_2CH_2CH_3$), 1.55 (1H, m, *CH*$_2$$CH_2CH_2CH_3$), 1.35 (4H, m, CH_2*CH*$_2$*CH*$_2$$CH_3$), 0.93 (3H, m, $CH_2CH_2CH_2$*CH*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 165.9, 155.7, 148.6, 143.3, 141.3, 132.1, 127.7, 127.1, 125.0, 120.6, 120.0, 118.4, 66.6, 65.2, 52.0, 47.3, 34.2, 27.7, 22.3, 13.9; HRMS (ESI) calcd for $C_{26}H_{29}NNaO_4$ $(MNa)^+$: 442.1989, found: 442.2021.

Allyl N-α-Fmoc-N-γ-trityl-acrylicglutamate. 90% yield as white solid; R_f=0.85 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3314.3, 3058.5, 2928.5, 1718.5, 1665.8, 1517.1, 1492.3, 1447.0 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.78 (2H, d, *J*=8.0 Hz, ArH), 7.60 (2H, m, ArH), 7.41 (2H, m, ArH), 7.23–7.43 (17H, m, ArH), 6.86 (1H, dd, *J*=16.0, 5.0 Hz, *CH*═CHCO), 6.80 (1H, s, NH), 5.96 (2H, m, CH_2═*CH*CH_2O and CH═*CH*CO), 5.27–5.39 (3H, m, *CH*$_2$═CHCH_2O and NH), 4.67 (2H, d, *J*=6.0 Hz, CH_2═CH*CH*$_2$O), 4.49 (1H, m, CH), 4.41–4.54 (2H, m, Fmoc–CH_2), 4.22 (1H, t, *J*=7.0 Hz, Fmoc–CH), 2.35 (2H, m, CH_2*CH*$_2$CO), 1.97 (1H, m, *CH*$_2$$CH_2CO$), 1.82 (1H, m, *CH*$_2$$CH_2CO$); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 171.1, 165.7, 156.0, 147.7, 144.5, 143.3, 141.3, 132.1, 128.7, 128.0, 127.7, 127.1, 125.1, 121.1, 119.98, 118.4, 70.7, 66.6, 65.3, 51.7, 47.32, 33.4, 29.2; HRMS (ESI) calcd for $C_{44}H_{40}N_2NaO_5(MNa)^+$: 699.2830, found: 699.2857.

Allyl N-α-Fmoc-N-ε-Boc-acryliclysinate. 83% yield as white solid; R_f=0.70 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3347.6, 2937.0, 1716.6, 1692.8, 1528.1, 1450.3 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.80 (2H, d, *J*=7.0 Hz, ArH), 7.62 (2H, d, *J*=7.0 Hz, ArH), 7.44 (2H, m, ArH), 7.35 (2H, m, ArH), 5.97 (2H, m, CH_2═*CH*CH_2O and CH═*CH*CO), 6.86 (1H, dd, *J*=10.5, 1.5 Hz, *CH*═CHCO), 5.37 (1H, dd, *J*=17.0, 1.5 Hz, *CH*═CHCO), 4.89 (1H, s, NH), 4.67 (2H, d, *J*=6.0 Hz, CH_2═CH*CH*$_2$O), 4.48 (2H, m, Fmoc–CH_2), 4.57 (1H, s, NH), 4.36 (1H, m, CH), 4.24 (1H, t, *J*=7.0 Hz, Fmoc–CH), 3.15 (2H, m, $CH_2CH_2CH_2$*CH*$_2$NH), 1.60 (2H, m, *CH*$_2$$CH_2CH_2CH_2$NH), 1.46 (13H, m, *t*Bu and CH_2*CH*$_2$*CH*$_2$$CH_2$NH); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 165.9, 156.2, 155.9, 148.5, 143.9, 143.8, 141.3, 132.1, 127.7, 127.1, 125.0, 124.8, 120.6, 120.0, 118.4, 79.1, 66.6, 65.2, 52.0, 47.2, 40.0, 33.8, 29.7, 28.4, 22.8; HRMS (ESI) $C_{31}H_{38}N_2NaO_6$ calcd for $(MNa)^+$: 557.2622, found: 557.2649.

3.2.7. Acrylic acid 10; general procedure for the allyl ester deprotections. To a stirring solution of the allyl ester (0.1 M) and $Pd(PPh_3)_4$ (0.1 equiv.) in CH_2Cl_2 was added *n*Bu_3SnH (5.0 equiv.). The reaction mixture was stirred at 23 °C 90 min. The crude reaction mixture loaded directly onto a silica gel column and eluted with MeOH–CH_2Cl_2.

N-Fmoc-O-tButyl-acrylicaspartic acid (**10-Asp**). 45% yield as white-yellow solid; R_f=0.31 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3398.5, 2976.6, 1721.4, 1542.9, 1411.3 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 7.89 (2H, d, *J*=7.0 Hz, ArH), 7.70 (2H, m, ArH), 7.62 (1H, d, *J*=8.5 Hz, NH), 7.41 (2H, m, ArH), 7.33 (2H, m, ArH), 6.51 (1H, dd, *J*=15.5, 5.5 Hz, *CH*═CHCO$_2$H), 5.77 (1H, d, *J*=15.5 Hz, CH═*CH*CO$_2$H), 4.48 (1H, m, CH), 4.30 (2H, m, Fmoc–CH_2), 4.23 (1H, t, *J*=7.0 Hz, Fmoc–CH), 2.48 (2H, m, CH_2CO), 1.37 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 169.8, 155.8, 144.28, 141.2, 129.4, 128.1, 127.5, 125.6, 121.8, 120.6, 120.5, 80.5, 66.0, 49.3, 47.1, 40.5, 28.1; HRMS (ESI) $C_{25}H_{27}NNaO_6$ calcd for $(MNa)^+$: 460.1731, found: 460.1730.

N-Fmoc-acrylicnorleucine (**10-Nle**). 86% yield as white solid; R_f=0.43 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3401.2, 2954.8, 1701.7, 1542.6, 1409.4 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 7.89 (2H, d, *J*=7.0 Hz, ArH), 7.72 (2H, d, *J*=7.0 Hz, ArH), 7.51 (d, *J*=8.5 Hz, 1H, NH), 7.41 (2H, m, ArH), 7.33 (2H, m, ArH), 6.53 (1H, dd, *J*=15.0, 6.0 Hz, *CH*═CHCO$_2$H), 5.76 (1H, d, *J*=15.0 Hz, CH═*CH*CO$_2$H), 4.30 (2H, m, Fmoc–CH_2), 4.23 (1H, m, Fmoc–CH), 4.06 (1H, m, CH), 1.48 (2H, m, *CH*$_2$$CH_2CH_2CH_3$), 1.27 (4H, s, CH_2*CH*$_2$*CH*$_2$$CH_3$), 0.86 (3H, s, $CH_2CH_2CH_2$*CH*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 156.1, 144.4, 144.3, 141.2, 141.1, 128.1, 127.5, 125.7, 125.6, 120.5, 65.8, 52.0, 47.2, 34.1, 28.2, 22.3, 14.5; HRMS (ESI) calcd for $C_{23}H_{25}NNaO_4$ $(MNa)^+$: 402.1676, found: 402.1679.

N-α-Fmoc-N-γ-trityl-acrylicglutamine acid (**10-Gln**). 89% yield as white-yellow solid; R_f=0.40 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3405.8, 3057.5, 1698.8, 1494.1, 1409.2 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 8.65 (1H, s, CO_2H), 7.95 (2H, m, ArH), 7.73 (2H, m, ArH), 7.56 (1H, d, *J*=8.5 Hz, NH), 7.42 (2H, m, ArH), 7.34 (2H, m, ArH), 7.23 (6H, m, ArH), 7.19 (9H, m, ArH), 6.51 (1H, dd, *J*=16.0, 6.0 Hz, *CH*═CHCO$_2$H), 5.77 (1H, d, *J*=16.0 Hz, CH═*CH*CO$_2$H), 4.23–4.32 (3H, m, Fmoc–CH and Fmoc–CH_2), 4.10 (1H, m, CH), 2.33 (2H m, CH_2*CH*$_2$CO), 1.68 (2H, m, *CH*$_2$$CH_2CO$); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 171.9, 145.4, 144.4, 144.3, 141.2, 141.1, 129.4, 129.0, 128.0, 127.9, 126.7, 125.7, 121.8, 120.6, 110.2, 69.6, 65.9, 51.8, 47.2, 33.2, 30.5; HRMS (ESI) calcd for $C_{41}H_{36}N_2NaO_5$ $(MNa)^+$: 659.2517, found: 659.2567.

N-α-Fmoc-N-ε-Boc-acryliclysine (**10-Lys**). 91% yield as white solid; R_f=0.23 (SiO_2, $CHCl_3$–MeOH 9:1); IR (KBr): 3338.4, 2934.2, 1701.5, 1526.2, 1409.8 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 7.89 (2H, d, *J*=7.5 Hz, ArH), 7.72 (2H, d, *J*=7.5 Hz, ArH), 7.51 (1H, d, *J*=8.5 Hz, NH), 7.42 (2H, m, ArH), 7.34 (2H, m, ArH), 6.78 (1H, m, NH), 6.55 (1H, dd, *J*=15.5, 6.0 Hz, *CH*═CHCO$_2$H), 5.77 (1H, d, *J*=15.5 Hz, CH═*CH*CO$_2$H), 4.30 (2H, m, Fmoc–CH_2), 4.23 (1H, m, Fmoc–CH), 4.06 (1H, m, CH), 2.90 (2H, m, $CH_2CH_2CH_2$*CH*$_2$NH), 1.48 (2H, m, *CH*$_2$$CH_2CH_2CH_2$NH), 1.37 (9H, m, *t*Bu), 1.27 (4H, m, CH_2*CH*$_2$*CH*$_2$$CH_2$NH); ^{13}C NMR (100 MHz, DMSO-d_6) *δ*: 156.1, 156.0, 144.34, 144.3, 141.2, 129.4, 128.1, 127.5, 125.7, 125.6, 121.8, 120.5, 77.8, 65.8, 52.1, 47.2, 40.2, 34.0, 29.7, 28.7, 23.4; HRMS (ESI) $C_{28}H_{34}N_2NaO_6$ calcd for $(MNa)^+$: 517.2309, found: 517.2284.

3.3. Alloc-protected PNA monomers 14

The reactions were carried out in parallel on an Argonaut Quest according to the following procedure. 2-Chlorotritylchloride resin (75–100 mesh, 1% DVB, 1.6 mmol/g,

2.0 equiv.) preswelled in CH_2Cl_2 was treated with a solution of acid **11** (1.0 equiv.) and Et*i*Pr$_2$N (1.0 equiv.) in CH_2Cl_2 (10 mL/g). After 3 h, the resin was filtered and washed (CH_2Cl_2), and the resins were treated with DBU (1.0 equiv.) in CH_2Cl_2 (10 mL/g) for 5 min to induce Fmoc deprotection. After washing (CH_2Cl_2), the resins were suspended in CH_2Cl_2, cooled to 0 °C and Et*i*Pr$_2$N (4.0 equiv.) and allyl chloroformate (3.0 equiv.) in CH_2Cl_2 as a 1 M CH_2Cl_2 solution were added. The reaction was carried out twice for 10 min at 0 °C then washed and cleaved with AcOH–CF_3CH_2OH–CH_2Cl_2 (1:1:8) (5 mL/g of resin) for 1 h to obtain **14** which were precipitated in diethyl ether, pelletted by centrifugation (9000 g), resuspended in 1:1 MeCN–H_2O and lyophilized.

3.3.1. *N*-(2-Allyloxyaminoethyl)-*N*-(thymine-1-acetyl)-glycine (14-T). 60% yield as viscous oil; IR (KBr): 3422.0, 3054.7, 1707.7, 1540.9, 1474.2 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.32 (1H, m, C_6), 5.91 (1H, m, CH_2=*CH*CH_2O), 5.27 (1H, dd, J=17.0, 1.5 Hz, *CH_2*=$CHCH_2O$), 5.18 (1H, m, *CH_2*=$CHCH_2O$), 4.66 (1H, CH_2=CH*CH_2*O), 4.46–4.50 (3H, m, CH_2=CH*CH_2*O and CH_2CO), 4.08 (0.6H, s, *CH_2CO_2H*), 3.98 (1.4H, s, *CH_2CO_2H*), 3.44–3.10 (4H, m, CH_2CH_2), 1.17 (3H, s, Me); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.9, 167.6, 164.8, 156.4, 151.4, 142.5, 134.1, 117.5, 108.53, 64.9, 48.0, 47.5, 47.1, 44.6, 12.3; HRMS (ESI) calcd for $C_{15}H_{20}NaN_4O_7$ (MNa^+): 391.1224, found: 391.1187.

3.3.2. *N*-(2-Allyloxyaminoethyl)-*N*-[4-*N*-(benzhydryloxy-carbonyl)cytosine-1-acetyl]glycine (14-C). 46% yield as viscous oil; IR (KBr): 3404.5, 3066.7. 2947.0, 1719.7, 1561.6, 1499.9 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: rotamer (7.90, 7.87) (d, 1H, J=7.5 Hz, C_6), 7.45 (4H, m, ArH), 7.38 (4H, m, ArH), 7.30 (2H, m, ArH), 6.94 (1H, m, C_5), 6.81 (1H, s, *$CH(C_6H_5)_2$*), 5.91 (1H, m, CH_2=*CH*CH_2O), (5.27, 5.16) (2H, m, *CH_2*=$CHCH_2O$), (4.82, 4.62) (2H, s, CH_2CON), (4.50, 4.46) (2H, d, J=5.5 Hz, CH_2=CH*CH_2*O), (4.12, 3.99) (2H, s, N*CH_2CO_2H*), 3.11–3.47 (4H, m, NH*CH_2CH_2*N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: (171.4, 171.0), (168.0, 167.4), 163.5, 155.4, 152.8, 151.4, 151.4, 142.3, 140.8, 134.1, 128.0, 128.3, 126.9, 117.5, (94.2, 93.1), 77.9, 64.9, 64.7, 49.9, 48.2, 47.3, 43.4; HRMS (ESI) calcd for $C_{28}H_{29}N_5O_8$ (MH^+): 564.2089, found: 564.211.

3.3.3. *N*-(2-Allyloxyaminoethyl)-*N*-[6-*N*-(benzhydryloxy-carbonyl)adenine-9-acetyl]glycine (14-A). 58% yield as viscous oil; IR (KBr): 3419.4, 3068.7, 2935.0, 1718.6, 1522.2, 1467.1 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.60 (1H, s, C_2), 8.34 (1H, s, C_8), 7.54 (4H, d, J=7.5 Hz, ArH); 7.39 (4H, t, J=7.5 Hz, ArH), 7.30 (2H, t, J=7.5 Hz, ArH), 6.83 (1H, s, O*$CH(C_6H_5)_2$*), 5.91 (1H, m, CH_2=*CH*CH_2O), rotamer (5.36, 5.17) (s, 2H, N*CH_2*CON), (5.32, 5.27), (2H, m, *CH_2*=$CHCH_2O$), (4.53, 4.46) (2H, d, J=5.5 Hz, CH*CH_2*O), (4.33, 4.02) (2H, s, N*CH_2CO_2H*), 3.59–3.11 (4H, m, NH*CH_2CH_2*N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: (171.9, 170.8), (167.5, 167.0), (156.7, 156.4), 152.7, (151.9, 151.6), 149.7, (145.7, 145.6), 141.3, 134.1, 128.9, 128.1, 126.9, 123.0, 117.5, 117.4, 77.7, (65.0, 64.7), 49.8, (48.1, 47.3), (44.5, 44.3); HRMS (ESI) calcd for $C_{28}H_{30}N_7O_5$: ($M-CO_2+H^+$): 544.2303, found: 544.2254.

3.3.4. *N*-(2-Allyloxyaminoethyl)-*N*-[2-*N*-(benzhydryloxy-carbonyl)-guanine-9-acetyl]glycine (14-G). 61% yield as viscous oil; IR (KBr): 3377.8, 3246.0, 3066.7, 2944.9, 1731.6, 1486.3 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ rotamer (8.35, 8.33, 7.83, 7.81) (1H, s, C_8), 7.46 (4H, d, J=7.0 Hz, ArH); 7.37 (4H, t, J=7.5 Hz, ArH), 7.31 (2H, t, J=7.5 Hz, ArH), (6.88, 6.87, 6.80, 6.79) (1H, s, O*$CH(C_6H_5)_2$*), (6.01, 5.88) (1H, m, OCH_2*CH*=CH_2), (5.48- 5.42) (1H, m, NH), (5.36, 5.33, 5.30, 5.26) (1H, s, N*H*CH_2CH_2N), (5.27, 5.23, 5.18, 5.16) (1H, d, J=15.5 Hz, *CH_2*=$CHCH_2O$), (5.13, 4.21) (2H, m, N*CH_2*CON), (4.51, 4.46) (2H, m, CH_2=CH*CH_2*O), (4.84–4.45) (2H, m, CH*CH_2*CO), (4.30, 4.01) (2H, s, N*CH_2CO_2H*), 3.54–3.12 (4H, m, NH*CH_2CH_2*N); ^{13}C NMR (100 MHz, DMSO-d_6) δ: (171.3, 170.9), (167.5, 166.9), 156.7, 155.5, (154.2, 154.1), 149.8, 147.5, (140.9, 140.4), 134.0, (129.0, 128.9), 128.4, 126.9, (120.2, 120.0), 119.6, (117.6, 117.4), 78.5, (65.0, 64.7), 48.3, 47.3, 44.3; HRMS (ESI) calcd for $C_{28}H_{29}N_7O_6$: ($M-CO_2+H^+$): 560.2252, found: 560.2206.

3.4. Alloc-protected amino acids 25

The reactions were carried out in parallel on an Argonaut Quest according to the following procedure. To 2-Chlorotritylchloride resin (75–100 mesh, 1% DVB, 1.6 mmol/g, 1.0 equiv.) swelled in CH_2Cl_2 (5 mL/g) was added a solution of acid (1.4 equiv.) and Et*i*Pr$_2$N (2.0 equiv.) in CH_2Cl_2 (5 mL/g of resin). After 3 h, the resin was filtered and washed (CH_2Cl_2) and the Fmoc was removed by standard piperidine treatment (20% in DMF, 1.5 mL, 10 min). After washing, the resins were suspended in CH_2Cl_2 (5 mL/g), cooled to 0 °C and treated sequentially with Et*i*Pr$_2$N (4.0 equiv.) followed by allyl chloroformate (3.0 equiv.) as 1M solution in CH_2Cl_2. The reaction was repeated once for 10 min before washing and cleaving with TFA (2.5% in CH_2Cl_2, 10 mL/g) during 15 min. The filtrates were diluted with toluene (1:1 v:v), concentrated then lyophilised from AcCN–H_2O.

3.4.1. Alloc-Ala-OH. 72% yield as viscous oil; IR (KBr): 3328.5, 2994.9, 1714.0, 1537.7, 1455.8 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.69 (1H, bs, CO_2H), 6.77 (1H, s, NH), 5.93 (1H, ddt, J=17.0, 10.5, 5.0 Hz), 5.34 (1H, dd, J=17.0, 1.5 Hz, *CH_2*=$CHCH_2O$), 5.25 (1H, d, J=10.5 Hz, *CH_2*=$CHCH_2O$), 4.61 (1H, d, J=5.0 Hz, CH_2=CH*CH_2*O), 4.43 (1H, m, CH), 1.49 (3H, d, J=7.0 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.5, 155.8, 132.4, 118.0, 66.5, 49.4, 18.3; HRMS (ESI) calcd for $C_7H_{11}NaNO_4$ $(MNa)^+$: 196.0580, found: 196.0588.

3.4.2. Alloc-Arg(Pfb)-OH. 54% yield as viscous oil; IR (KBr): 3341.9, 2982.9, 1670.1, 1576.1, 1457.7; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.88 (1H, bs, CO_2H), 7.60 (1H, s, NH), 6.50 (1H, s, NH), 6.16 (1H, s, NH), 5.87 (1H, m, CH_2=*CH*CH_2O), 5.29 (1H, d, J=17.0 Hz, *CH_2*=$CHCH_2O$), 5.20 (1H, d, J=10.0 Hz, *CH_2*=$CHCH_2O$), 4.54 (2H, s, CH_2=CH*CH_2*O), 4.28 (1H, s, CH), 3.32 (2H, m, CH_2CH_2*CH_2*NH), 3.00 (2H, s, *$CH_2C(CH_3)_2$*), 2.51 (3H, s, ArMe), 2.47 (3H, s, ArMe), 2.12 (3H, s, ArMe), 1.91–1.73 (4H, s, *$CH_2CH_2CH_2$*NH), 1.50 (6H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.9, 169.2, 162.7, 156.9, 137.9, 134.7, 132.3, 129.0, 128.2, 125.3, 117.8, 87.6, 66.2, 53.2, 42.9, 41.3, 29.2, 28.5, 24.0, 19.2, 12.4; HRMS (ESI) calcd for $C_{23}H_{36}N_4O_7S$ $(MH)^+$: 511.2221, found: 511.2192.

3.4.3. Alloc-Asp(*t*Bu)-OH. 84% yield as viscous oil; IR (KBr): 3341.9, 2982.0, 1728.0, 1519.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.87 (1H, bs, CO_2H), 5.91 (1H, ddt, J=17.0, 10.0, 5.0 Hz, CH_2=$CH$$CH_2O$), 5.84 (1H, d, J=8.0 Hz, NH), 5.33 (1H, dd, J=17.0, 1.5 Hz, CH_2=$CHCH_2O$), 5.23 (1H, dd, J=10.0, 1.5 Hz, CH_2=$CHCH_2O$), 4.61 (3H, m, CH_2=$CHCH_2O$ and CH), 2.98 (1H, dd, J=17.0, 5.0 Hz, CH_2O*t*Bu), 2.79 (1H, dd, J=17.0, 5.0 Hz, CH_2O*t*Bu), 1.45 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 181.8, 176.3, 162.2, 138.5, 124.1, 88.4, 72.3, 56.5, 43.8, 34.1; HRMS (ESI) calcd for $C_{12}H_{19}NaNO_6$ $(MNa)^+$: 296.11045, found: 296.1119.

3.4.4. Alloc-His(Boc)-OH. 85% yield; IR (KBr): 3329.9, 2935.0, 1720.1, 1528.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18 (1H, s, CHNCHN), 7.25 (1H, s, CHNCHN), 5.95 (1H, m, CH_2=$CH$$CH_2O$), 5.86 (1H, d, J=10.5 Hz, NH), 5.35 (1H, d, J=17.0 Hz, CH_2=$CHCH_2O$), 5.25 (1H, d, J=10.5 Hz, CH_2=$CHCH_2O$), 4.61 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 4.56 (1H, m, CH), 3.14 (2H, m, $CHCH_2C$), 1.63 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$): 173.0, 162.3, 155.7, 146.2, 136.8, 132.7, 117.8, 115.5, 86.6, 65.7, 53.2, 29.7, 27.8; HRMS (ESI) calcd for $C_{15}H_{22}N_3O_6$ $(MH)^+$: 340.1503, found: 340.1513

3.4.5. Alloc-Lys(Boc)-OH. 91% yield as viscous oil; IR (KBr): 3346.0, 2935.0, 1701.0, 1522.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.36 (1H, s, NH), 5.91 (1H, m, CH_2=$CH$$CH_2O$), 5.73 (1H, d, J=8.0 Hz, NH), 5.31 (1H, d, J=17.0 Hz, CH_2=$CHCH_2O$), 5.22 (1H, d, J=10.0 Hz, CH_2=$CHCH_2O$), 4.58 (2H, d, J=5.0 Hz, CH_2=$CHCH_2O$), 4.37 (1H, m, CH), 3.11 (2H, m, $CH_2CH_2CH_2CH_2NH$), 1.88 (1H, m, $CH_2CH_2CH_2CH_2NH$), 1.76 (1H, m, $CH_2CH_2CH_2CH_2NH$), 1.45 (13H, s, *t*Bu and $CH_2CH_2CH_2CH_2NH$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.9, 175.5, 156.2, 132.6, 117.8, 79.6, 65.9, 53.6, 40.0, 31.8, 29.5, 28.4; HRMS (ESI) calcd for $C_{15}H_{26}NaN_2O_6$ $(MNa)^+$: 353.1683, found: 353.1705.

3.4.6. Alloc-Nle-OH. 73% yield as viscous oil; IR (KBr): 3329.9, 2959.1, 1715.5, 1537.8, 1456.4 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.47 (1H, bs, CO_2H), 6.34 (1H, d, J=8.5 Hz, NH), 5.95 (1H, ddt, J=16.5, 10.5, 5.5 Hz), CH_2=$CH$$CH_2O$), 5.34 (1H, dd, J=16.5, 1.5 Hz, CH_2=$CHCH_2O$), 5.25 (1H, dd, J=10.5, 1.5 Hz, CH_2=$CHCH_2O$), 4.61 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 4.39 (1H, m, CH), 1.89 (1H, m, $CH_2CH_2CH_2CH_3$), 1.73 (1H, m, $CH_2CH_2CH_2CH_3$), 1.37 (4H, m, $CH_2CH_2CH_2CH_3$), 0.93 (3H, t, J=7.0 Hz, $CH_2CH_2CH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.5, 156.0, 132.5, 118.0, 53.7, 66.0, 32.0, 27.2, 22.2, 13.8; HRMS (ESI) calcd for $C_{10}H_{17}NaNO_4$ $(MNa)^+$: 238.1049, found: 238.1035.

3.4.7. Alloc-Phe-OH. 84% yield as viscous oil; IR (KBr): 3329.9, 3042.7, 2947.0, 1718.6, 1522.2, 1492.6 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.32 (3H, m, ArH), 7.21 (2H, m, ArH), 6.27 (1H, d, J=8.0 Hz, NH), 5.91 (1H, ddt, J=17.0, 10.5, 5.5 Hz, CH_2=$CH$$CH_2O$), 5.26 (2H, m, CH_2=$CHCH_2O$), 4.72 (1H, dt, J=8.0, 6.0 Hz, CH), 4.59 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 3.24 (1H, dd, J=14.0, 6.0 Hz, CH_2), 3.14 (1H, dd, J=14.0, 6.0 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.3, 155.8, 135.5, 132.4, 129.3, 128.7, 127.3, 118.0, 66.0, 54.5, 37.7; HRMS (ESI) calcd for $C_{13}H_{15}NaNO_4$ $(MNa)^+$: 272.0893, found: 272.0910.

3.4.8. Alloc-Pro-OH. 84% yield as viscous oil; IR (KBr): 3102.6, 1704.3, 1450.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.59 (1H, bs, CO_2H), 5.90 (1H, m, CH_2=$CH$$CH_2O$), 5.34 (2H, m, CH_2=$CHCH_2O$), 4.65 (2H, m, CH_2=$CHCH_2O$), 4.41 (1H, m, N$CH$$CO_2H$), 3.55 (2H, m, N$CH_2CH_2CH_2$), 2.20 (2H, m, $NCH_2CH_2CH_2$), 1.98 (2H, m, $NCH_2CH_2CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: (155.7, 154.5), (177.9, 176.4), 132.5, (117.7, 117.3), (66.5, 66.2), (59.2, 58.6), (46.9, 46.6), (30.9, 29.4), (24.3, 23.4); HRMS (ESI) calcd for $C_9H_{14}NO_4$ $(MH)^+$: 200.0917, found: 200.0898.

3.4.9. Alloc-Ser(*t*Bu)-OH. 70% yield as viscous oil; IR (KBr): 3331.5, 2976.2, 1729.8, 1517.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.93 (1H, ddt, J=17.0, 10.5, 5.5 Hz, CH_2=$CH$$CH_2O$), 5.65 (1H, d, J=8.5 Hz, NH), 5.35 (1H, d, J=17.0 Hz, CH_2=$CHCH_2O$), 5.25 (1H, d, J=10.5 Hz, CH_2=$CHCH_2O$), 4.62 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 4.49 (1H, m, CH), 3.91 (1H, dd, J=9.0, 3.5 Hz, CH_2O*t*Bu), 3.61 (1H, dd, J=9.0, 3.5 Hz, CH_2O*t*Bu), 1.19 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.1, 156.1, 132.5, 118.0, 74.0, 66.0, 61.7, 54.2, 27.2; HRMS (ESI) calcd for $C_{11}H_{19}NaNO$ $(MNa)^+$: 268.1155, found: 268.1135.

3.4.10. Alloc-Val-OH. 85% yield as viscous oil; IR (KBr): 3329.9, 2970.9, 1707.6, 1534.3, 1468.3 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.34 (1H, d, J=8.5 Hz, NH), 5.95 (1H, ddt, J=17.0, 10.5, 5.5 Hz, CH_2=$CH$$CH_2O$), 5.34 (1H, dd, J=16.0, 1.0 Hz, CH_2=$CHCH_2O$), 5.25 (1H, dd, J=10.5, 1.0 Hz, CH_2=$CHCH_2O$), 4.61 (2H, d, J=5.5 Hz, CH_2=$CHCH_2O$), 4.36 (1H, dd, J=9.0, 4.4 Hz, CH), 2.26 (1H, m, $CH(CH_3)_2$), 1.03 (3H, d, J=7.0 Hz, $CH(CH_3)_2$), 0.96 (3H, d, J=7.0 Hz, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ:176.9, 156.3, 132.5, 118.0, 58.8, 66.1, 31.0, (18.99, 17.32); HRMS (ESI) calcd for $C_9H_{15}NaNO_4$ $(MNa)^+$: 202.1074, found: 202.1082.

3.5. Library synthesis

Rink amide resin (0.8 mmol/g) was loaded with a substoichiometric amount of *N*-α-Fmoc-*N*-ε-4-methyltrityl-lysine (0.2 mmol/g), DIC (1.0 equiv.), HOBt (1.0 equiv.) in DMF (10 mL/g) for 12 h and acetic anhydride (5.0 equiv.) with 2,6-lutidine (5.0 equiv.) was added to cap the resin (2 h). Four 280 mg portions of this resin were distributed into 5 mL tubes on an Argonaut Quest 210 and, after 30 min swelling in CH_2Cl_2, the resins were deprotected with 20% piperidine in DMF (3 mL) for 2.5 min. An automated washing procedure was used after each step involving four DMF washes (2 mL/g of resin) with a 30 s agitation (no contracting solvents such as methanol or diethyl ether were used). The resins were then treated with Fmoc-8-amino-3,6-dioxaoctanoic acid (2.0 equiv.), HOBt (4.0 equiv.) and DIC (4.0 equiv.) in DMF (2 mL) for 6 h. Subsequent Fmoc deprotection (20% piperidine in DMF, 3 mL) followed by coupling of each pool to an acrylic amino acids **10** (2.0 equiv.), DIC (4.0 equiv.), HOBt (4.0 equiv.) afforded four pools of intermediates **21**. The Fmocs were removed (20% piperidine in DMF, 3 mL) and each pool, in CH_2Cl_2 at 0 °C, was treated sequentially with Et*i*Pr_2N (4.0 equiv.) and Alloc-Cl (4.0 equiv.) for 30 min. The pools were then treated with a 1% TFA solution containing 5% Et_3SiH for 1 h to obtain the

polymer bound intermediates **23** which were encoded according to the general procedure below. The four pools were then combined for the Alloc deprotection according to the general procedure below and redistributed in ten 5 mL tubes. All resin manipulations and distributions were carried out as slurries using an isopycnic mixture of CH_2Cl_2 and DMF and an eppendorf repeater for distribution. The ten pools were subjected to their respective amino acid coupling followed by the encoding according to the general procedures to obtain ten pools of **28**. Two more iteration of this cycle led to 10 pool of intermediate **30** which were mixed, and Fmoc deprotected (20% piperidine) followed by treatment of fluoresein isothiocyanate (4.0 equiv.) and 2,6-lutidine in DMF (10 mL/g) for 5 h while shielding the reaction from light with aluminum foil to obtain after filtration and washing a distinctly yellow/orange polymer. This resin was treated with TFA–cresol (4:1, 10 mL) for 3 h then precipitated in Et_2O (200 mL) and pelleted by centrifugation (9800 g). The pellet was taken back up in neat TFA (7 mL) and reprecipitated and pelleted in Et_2O (200 mL). The pellet was washed several times with Et_2O than dissolved in a 1:1 mixture of AcCN–H_2O (1:1, 20 mL) and lyophilized to obtain 390 mg of a bright yellow powder.

3.5.1. General procedure for encoding steps. The resin was treated with 20% piperidine in DMF for 2.5 min, washed according to the previously described procedure and treated with a premixed (5 min) solution of PNA monomer **11** (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), HATU (3.5 equiv.) and 2,6-lutidine (6.0 equiv.) for 1 h. The coupling was repeated a second time then the resin was capped with acetic anhydride (5.0 equiv.) and 2,6-lutidine (5.0 equiv.) in DMF (10 mL/g). This cycle was repeated 4 times for the first and last codons (**23** to **27** and **29** to **30**) and 3 times for the second and third codons (**27** to **28** and **28** to **29**).

3.5.2. General procedure for Alloc deprotection. The resin was treated with a solution of $Pd(PPh_3)_4$ (0.2 equiv.), AcOH (10 equiv.) in CH_2Cl_2 (5 mL/g) followed by a solution of Et_3SiH in DMF (5 mL/g) for 30 min.

3.5.3. General procedure for DIC-mediated couplings. A solution of acid **25**, DIC (4.0 equiv.) and HOBt (4.0 equiv.) in DMF (10 mL/g of resin) was premixed for 5 min than added to the resin and the reaction was continued for 2 hr then capped by the addition of acetic anhydride (5.0 equiv.) and 2,6-lutidine (5.0 equiv.).

4. Supplementary Material

Mass spectroscopy data of compounds **27–29** as well as other library intermediates.

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