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Synthesis of (*R*)- and (*S*)-Fmoc-Protected Diethylene Glycol Gamma PNA Monomers with High Optical Purity

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Abstract

A robust synthetic route has been developed for preparing optically-pure, Fmoc-protected diethylene glycol-containing (*R*)- and (*S*)-γPNA monomers. The strategy involves the application of 9-(4-bromophenyl)-9-fluorenyl as a temporary, safety-catch protecting-group for the suppression of epimerization in the *O*-alkylation and reductive amination steps. The optical purities of the final monomers were determined to be greater than 99.5% ee, as assessed by ^{19}F-NMR and HPLC. The new synthetic methodology is well-suited for large-scale monomer production, with most synthetic steps providing excellent chemical yields without the need for chromatographic purification other than a simple workup and precipitation.

Introduction

Synthetic oligonucleotides are valuable as molecular tools and reagents for biology, biotechnology, and medicine, as well as for molecular engineering and computation.[1-4] Paramount to the success of many of these applications is a reliance on their ability to recognize and bind the designated DNA or RNA targets, or their obligatory partners with high affinity and sequence-specificity. Other desirable biophysical attributes, *e.g.* cellular transduction, biodistribution, metabolism, and clearance may be necessary for intracellular and *in vivo* applications; however, they are not required for *in vitro* experiments. It is therefore essential to be able to modify the structures and chemical functionalities of these synthetic oligonucleotide molecules with ease, so that they can be tailor-made to meet the specific requirements in hand, since, in general, there is no one particular nucleic acid system or chemical outfit that can fulfill all the application requirements.

A particular class of nucleic acid mimic that is endowed with synthetic flexibility is peptide nucleic acid (PNA). Developed by Nielsen and coworkers[5] in the early 1990's, PNA comprises an entirely different backbone skeleton from that of DNA or RNA, made up of *N*-(2-aminoethyl) glycine instead of the usual sugar phosphodiester units. PNA has several appealing features, including tight and sequence-specific binding of DNA or RNA, and resistance to proteolytic and nucleolytic degradation.[6] Furthermore, the acyclic and achiral backbone provides a convenient means for installing new chemical groups or for making structural modifications. However, PNA is not without shortcomings. The charge-neutral polyamide backbone that confers PNA with many of its attractive features renders it less soluble in water than the natural counterparts. As a consequence, PNA has a tendency to aggregate and adhere to surfaces and other macromolecules in a non-specific manner.[7,8] While this issue has been addressed to some degree,[9-11] further improvements in this and other areas, including synthetic methodology and

recognition property, are warranted in order to expand the scope and utility of PNA in the biological and biomedical arenas.

A promising approach involves installation of a chiral center at the gamma backbone.[12-20] The presence of a chiral center at this position has been shown to transform PNA from a random-fold into either a right-handed (RH) or a left-handed (LH) helical motif depending on the stereochemistry.[21,22] The LH conformer is unable to hybridize to RH, or to DNA or RNA, due to conformational mismatch. In contrast, due to the complementarity in conformation, the RH conformer hybridizes to DNA or RNA with exquisite affinity and sequence-specificity, and is the only class of oligonucleotide molecules developed to date that has been shown to be capable of invading double helical B-form DNA (B-DNA) without sequence limitation.[23] Although locked nucleic acid (LNA) has been reported to be able to invade DNA, strand invasion requires simultaneous binding of both strands of DNA double helix. Such a demand has practical limitations.[24] Furthermore, by incorporating a diethylene glycol (commonly referred to as miniPEG, or MP) moiety at the gamma backbone, the water solubility and biocompatibility of PNA can be significantly improved without the need to introduce charged groups,[25] which can compromise the binding affinity and/or specificity of the oligonucleotide molecule. These newly endowed properties of PNA have been exploited in a number of applications including biosensing,[26-28] gene editing,[29,30] and molecular engineering.[22] The challenge with this undertaking, however, is in the production of chemical building blocks in large scale and with high optical purity, and ones that are compatible with automated solid-phase peptide synthesis (SPPS). The former is essential, since the presence of even a trace amount of enantiomeric impurity could have profound adverse effects on the conformation and hybridization property of oligomer. The previously reported Boc-chemistry is not ideally-suited for automated SPPS due to the strong acid (TFA) typically employed to remove Boc group, which is conducive to acid corrosion and rapid deterioration of the instrument.[25] Here we report the development of a

robust method for scaling up the production of enantiomerically-pure Fmoc-protected (*R*)- and (*S*)-MPγPNA monomers that are suitable for automated synthesis from relatively inexpensive starting materials (Chart 1).

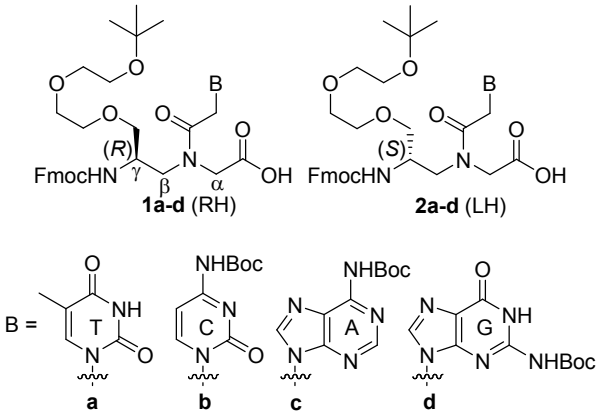
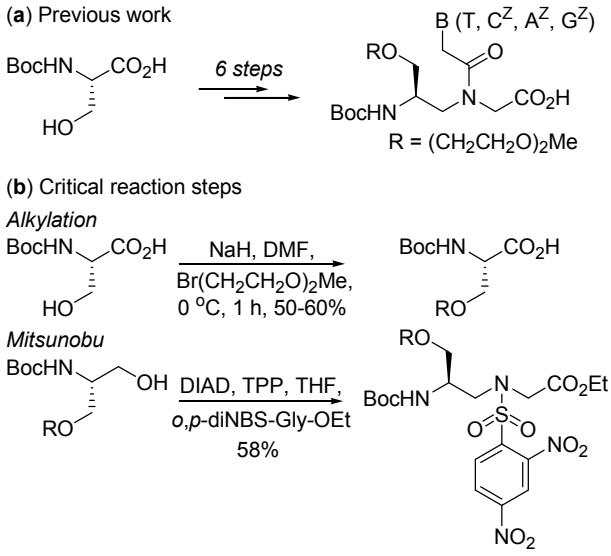


Chart 1

Results and Discussion

Rationale. To date most chiral PNA monomers were prepared through the reductive amination route, starting with Boc- or Fmoc-protected amino acids.[31] Such a coupling reaction, however, is known to produce configurationally unstable products due to the propensity of Boc- and Fmoc-aminoaldehyde intermediates to undergo epimerization even under mild experimental conditions.[32-35] Previously, we were able to minimize the extent of epimerization at gamma(γ)-carbon by carrying out *O*-alkylation of the serine sidechain under a strict reaction condition and by employing Mitsunobu reaction in the preparation of backbone (Scheme 1).[25] We managed to produce small quantities of Boc-protected backbone and the corresponding Boc-MPγPNA monomers for the initial proof-of-concept studies (Scheme 1a). However, it is daunting to scale up monomer production *via* this synthetic route without compromising optical purity. One reason

is that the alkylation step is performed at a relatively low temperature and for a short reaction time to minimize the degree of epimerization at the expense of chemical yield, and another reason is the difficulty in removing triphenylphosphine oxide byproduct in the Mitsunobu reaction from the desired backbone (Scheme 1b). To circumvent the shortcomings of scalability and optical purity, and the harsh reaction conditions employed in Boc-chemistry, we examined several amino-protecting groups including trityl (Trt),[36,37] fluorenylmethyloxycarbonyl (Fmoc),[38] dibenzyl,[39] 9-phenylfluorenyl (PhF),[40] and 9-(4-bromophenyl)-9-fluorenyl (BrPhF),[41] along with numerous synthetic routes. Among them we found BrPhF to be the most robust temporary protecting group, and the synthetic route outlined in Scheme 2 and 4 to be the most facile in the production of Fmoc-MPγPNA monomers **1a**-**d** and **2a**-**d** with high optical purity (Chart 1). The other protecting groups were not as successful due to several setbacks as outlined in Scheme S1 and S2 (Supplemental Information).



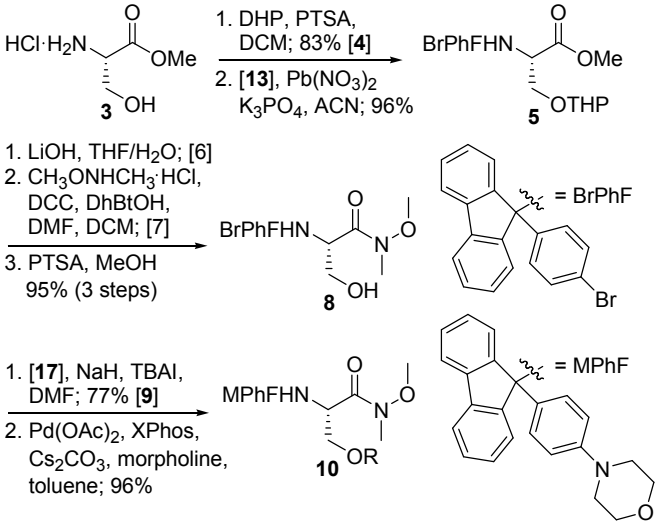
Scheme 1

Choice of PhF as a temporary protecting group. Previously we have shown that PhF, an amino-protecting group originally developed by Lubell and Rapoport[42] in the early 1980's, was

effective in shielding the α-center of amino acids against epimerization under highly basic conditions.[43] The protective capacity of PhF has been largely attributed to its sterically imposing size and preference as a leaving group. The demanding size forces the α-proton of ester to be in an unfavorable orientation for deprotonation. Under a harsh deprotonation condition, such as treatment with base under refluxing condition, the reprotonation of α-anion is significantly slower than the elimination of PhF. This preserves the stereochemistry of the intact PhF-protected ester, whereas the eliminated byproduct can be readily removed with aqueous workup.[42] However, we found PhF to be difficult to remove in the subsequent steps, requiring either a strong acid or a prolonged hydrogenolysis. The former is incompatible with other protecting groups, while the latter could lead to partial reduction of the cytosine nucleobase.[44] To obviate this challenge we adopted the halogenated derivative, namely BrPhF, as reported by Surprenant and Lubell,[45] which retains the full protection of PhF but that can be selectively removed under a mild acidic condition following the insertion of an electron-donating group, such as morpholine.

The strategy for incorporating BrPhF is outlined in Scheme 2. The sidechain of *L*-serine **3** was initially protected with tetrahydropyranyl acetal (THP) group, followed by *N*-alkylation with BrPhF. We found THP protection,[46] instead of the reported *in situ* trimethylsilylation,[40] to be necessary in providing high yield in the preparation of BrPhF-*L*-Ser(THP)-OMe **5**. The conversion of **5** to Weinreb amide **8** was performed in three steps: hydrolysis, amidation, and THP removal, with 95% overall yield. Such a reaction sequence appears lengthy and could potentially be replaced by a single transformation step by employing a Lewis acid, such as Me_2AlCl, and *N*,*O*-dimethylhydroxylamine hydrochloride;[47] however, we found such a reaction to be low yielding and not reproducible, making it difficult to scale up in comparison to those shown in Scheme 2. *O*-alkylation of **8** with MP-sidechain followed by Buchwald-Hartwig amination yielded the acid-labile MPhF-protected Weinreb amide **10**. For optimization purpose, we

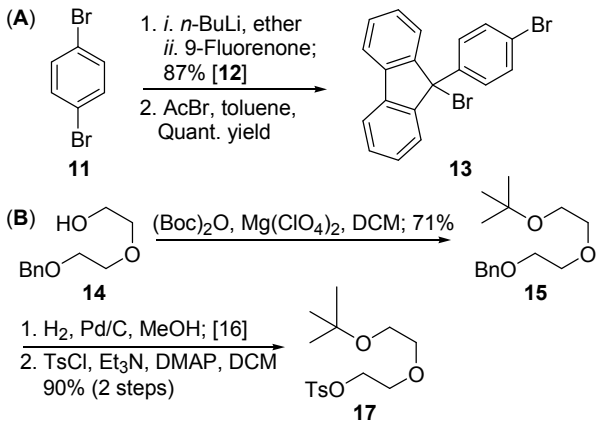
screened several reagents and reaction conditions (Table S1, Supplemental Information), and found Cs_2CO_3 and XPhos to be the best combination. Overall, compound **10** was prepared in seven steps with greater than 56% overall yield, with several reaction steps carried out in nearly quantitative yields without purification.



Scheme 2

Preparation of BrPhF and MP-sidechain. The required BrPhFBr precursor **13** and tosylated MP-sidechain **17** were prepared as shown in Scheme 3A and B, respectively. Treatment of 1,4-dibromobenzene with 9-fluorenone in the presence of *n*-BuLi gave BrPhF-OH **12** in good yield. Bromination of this compound with acetyl bromide in toluene provided **13** with quantitative yield.[48] We have also attempted the HBr aqueous condition as reported by Christie and Rapoport,[49] but we found it to be relatively inefficient due to the rapid solvolysis of the target compound **13**. The MP-sidechain precursor **17** was prepared from a commercially available benzyl diethylene glycol in three steps: conversion of alcohol to *t*-butyl ether, removal of Bn group, and tosylation. The first step was carried out in accordance with the protocol reported by

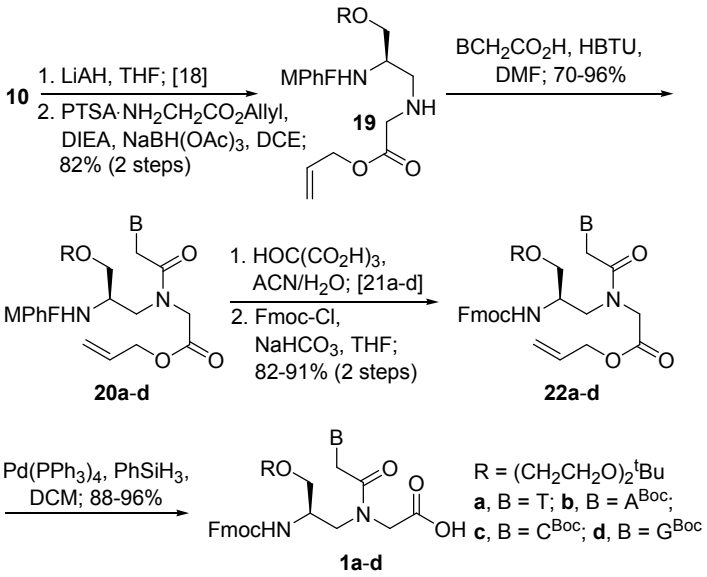
Bartoli and Sambri,[50] involving a simple treatment with magnesium perchlorate and Boc-anhydride in DCM. Hydrogenation of **15** followed by tosylation yielded the desired MP-sidechain precursor **17**. This route is much simpler to install *t*-butyl ether than the conventional method, which requires harsh conditions employing isobutylene in the presence of a strong acid catalyst.[51]



Scheme 3

Synthesis of backbones and monomers. The backbone intermediates, both (*R*)- and (*S*)-configuration, were prepared in the same way starting from *L*- and *D*-serine, respectively. However, only preparation of the *L*-configuration is shown (Scheme 4). Conversion of Weinreb amide **10** to an aldehyde followed by standard reductive amination[52] yielded the desired backbone **19** with high optical purity, as shown in the section below. Coupling the backbone with Boc-protected carboxymethylnucleobases (T, C^{Boc}, A^{Boc}, and G^{Boc})[44,53] yielded the respective monomer esters **20a**-**d**. Removal of MPhF was accomplished by treatment with citric acid without affecting Boc, *t*-butyl ether, or allyl protecting group, as assessed by ESI-MS (Figure S28 and S29). We have also assessed the dichloroacetic acid/triethylsilane condition as reported by Surprenant and Lubell,[45] but found that such a reaction condition led to the removal

of both MPhF and Boc protecting groups. *In situ* Fmoc protection, followed by removal of the allyl group under a neutral condition afforded the right-handed (*R*)-MPγPNA monomers **1a**-**d**.



Scheme 4

Assessment of optical purity. The optical purities of monomers were determined by ^{19}F-NMR and HPLC following the conversion of monomers **1a** (RH) and **2a** (LH) to the respective Mosher derivatives **23** and **24**. It is interesting to note that in this case ^{19}F-NMR spectra showed a single peak for compound **23** and a doublet for **24** (Figure 1). The extra peak in **24** is not due to the presence of a racemate, but rather due to the compound existing in two rotameric forms, as documented in the literature.[25] The difference in the ^{19}F-NMR patterns is attributed to the difference in the local chemical environments of the two compounds. Taking the difference in the mole fraction of the two enantiomers, as determined by the peak areas, yielded an enantiomeric excess (ee) greater than 99.5% for both monomers **1a** and **2a**.[54] This finding was further substantiated by HPLC analysis (Figure 2), which showed no trace evidence of the racemate present in these monomers, with an ee value determined to be at least 99.5%. The

broader shoulders observed in each trace could be due to the presence of chemical purities since the samples were not column-purified; they were injected into HPLC as crude mixtures. Collectively, these results demonstrate the effectiveness of BrPhF and MPhF in suppressing epimerization in the two critical reaction steps, *O*-alkylation of serine sidechain and reductive amination, in the preparation of Fmoc-MPγPNA backbones and the corresponding monomers—even under a relatively harsh condition, treatment with base at an elevated temperature, carried out in the alkylation step to drive the reaction to completion.

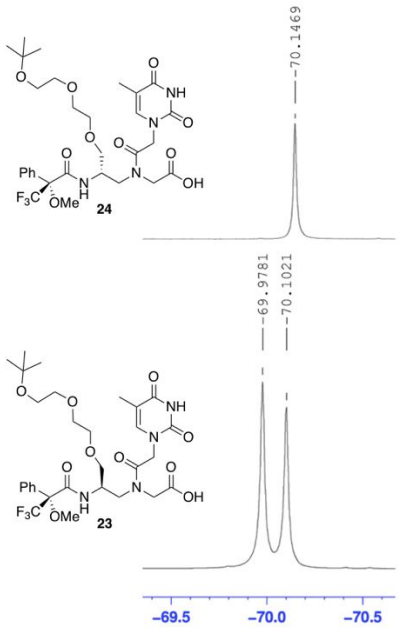


Figure 1. ^{19}F-NMR spectra of compounds **23** and **24**. The doublet pattern observed with compound **23** is the result of the formation of rotamers in DMSO-d_6.

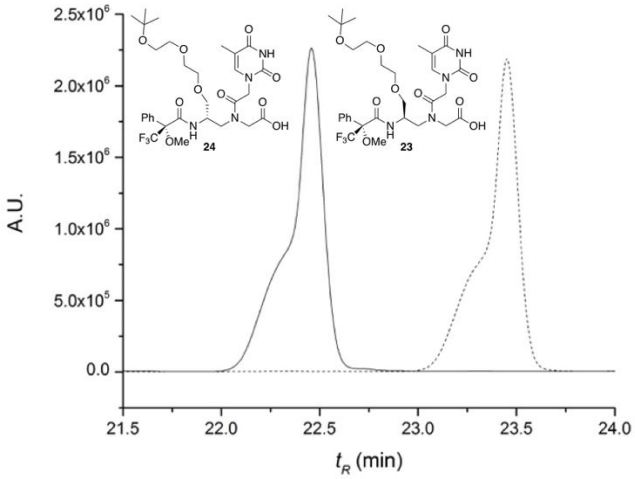


Figure 2. HPLC chromatograms of compounds **23** and **24**. Overlapping peaks, an indication of the presence of racemate, were not observed. HPLC condition: C18 column (dimensions 4.6 mm X 250 mm), 1 mL/min flow rate, 60 ^{o}C oven temperature, 25 to 75% ACN/H_2O with 0.1% TFA in 30 min.

Conclusion

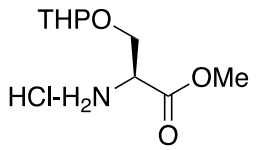
In summary, we have shown that optically-pure (*R*)- and (*S*)-Fmoc-MPγPNA monomers could be prepared in large scales from relatively inexpensive *L*- and *D*-serine, respectively. The new methodology employed the safety-catch BrPhF-protecting group and its morpholine derivative, namely MPhF, in shielding the α-carbon from undergoing epimerization in the *O*-alkylation and reductive amination steps. The ee values of monomers produced by this synthetic route were determined to be greater than 99.5%, as assessed by ^{19}F-NMR and HPLC. Although the synthetic route is relatively lengthy in comparison to the published report,[25] many of these reaction steps provided excellent chemical yields and were used in the subsequent steps without purification. Upon the insertion of morpholine, the resulting MPhF-protecting group can be removed under a mild acidic condition without affecting Boc, *t*-butyl ether, or allyl protecting group. The resulting amine can be readily re-protected with Fmoc or any other functional groups as desired. This is in direct contrast to all the other synthetic routes that have been examined,[25,43] which were prone to epimerization and required a strenuous chromatographic

purification in every step. The reported methodology is general and robust, and could be applied to the preparation of γPNA monomers with other chemical functionalities at the gamma backbone without the concern for erosion of optical purity, even under basic conditions at an elevated temperature. It should be pointed out that such a synthetic route may not be practical for the synthesis of monomers for basic research consumption due to its potentially high cost. However, it will be essential for the production of GMP-grade materials for clinical testing—for instance, in gene regulation and editing—where obtaining a single enantiomeric product in large scale is of paramount.

Experimental Section

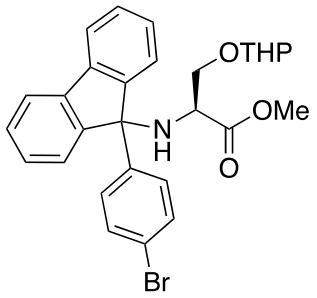
All commercial reagents were used without further purification. Solvents were dried by standard methods and distilled freshly prior to use. ^{1}H-, ^{13}C- and ^{19}F-NMR spectra were recorded on a Bruker 500.13 MHz Avance NMR spectrometer using standard Bruker software. The ^{1}H NMR spectra were referenced to DMSO-d_6 (2.50 ppm) or TMS in $CDCl_3$ (0.00 ppm). The $^{13}C\{^{1}H\}$ NMR spectra were referenced to $CDCl_3$ (77.23 ppm) or DMSO-d_6 (39.52 ppm). The ^{19}F NMR spectra were referenced to TFA in DMSO-d_6 (-76.55 ppm). Flash chromatography was performed using standard silica gel (60Å, 63-200 μm) or NH_2 silica gel (100 Å, 40-75 μm). TLC was generally performed with silica gel 60 F-254 precoated plates or NH_2 HPTLC plate. High Resolution Mass Spectrometry (HRMS) was performed with a Thermo Scientific Exactive Plus EMR Orbitrap ESI mass spectrometer. GC-MS analysis was performed on a Hewlett-Packard Agilent 6890-5973 GC-MS workstation with a Hewlett-Packard fused silica capillary column cross-linked with 5% phenylmethylsiloxane and Helium as the carrier gas. The following conditions were used for all GC-MS analyses: injector temperature, 250 °C; initial temperature, 70 °C; temperature ramp, 10 °C/min; final temperature, 280 °C. High-performance

liquid chromatography was performed on a Shimadzu UFLC system with a C18 column (dimensions 4.6 mm X 250 mm) and 1 mL/min flow rate. Gradients and temperature were indicated in the figure captions.



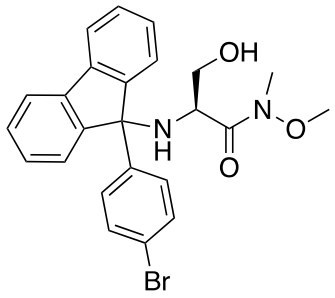
4

O^3*-(Tetrahydropyran-2-yl)-L-serine methyl ester hydrochloride* (**4**).[46] To a suspension of *L*-serine methyl ester hydrochloride **3** (19.5 g, 127.3 mmol) in anhydrous CH_2Cl_2 (100 mL) was added 3,4-dihydro-1-H-pyran (17.1 mL, 187.8 mmol) and *p*-toluenesulfonic acid monohydrate (0.476 g, 2.5 mmol). The reaction mixture was allowed to stir at room temperature for 20 h. The resulting mixture was chilled in an ice bath, filtered and washed with chilled CH_2Cl_2 to afford white powder without further purification (25.7 g, 83%). $C_9H_{18}ClNO_4$; white solid; TLC (MeOH/CH_2Cl_2, 10:90) R_f= 0.6; diastereomeric ratio: 83:17; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 8.77 (3H, s), 4.63–4.57 (1H, m), 4.33–4.28 (1H, m), 4.04–3.99 (1H, m), 3.82–3.75 (4H, m), 3.63–3.58 (1H, m), 3.46–3.44 (1H, m), 1.79–1.41 (6H, m); ^{13}C{^{1}H} (125.77 MHz, DMSO-d_6) δ 168.1, 98.3/97.7, 64.6/64.4, 61.3/61.0, 52.8, 52.3/52.2, 29.7/28.5, 24.8, 18.5. HRMS (ESI) calcd for $C_9H_{18}NO_4$: 204.1236; found: *m*/*z* 204.1223 [M – HCl + H]$^+$.



5

N-(9-(4-Bromophenyl)-9-fluorenyl)-O³-(tetrahydropyran-2-yl)-serine methyl ester (**5**). To a solution of serine ester **4** (8.00 g, 33.4 mmol) in anhydrous ACN (110.0 mL) was added $Pb(NO_3)_2$ (55.32 g, 167.0 mmol) and K_3PO_4 (35.5 g, 167.0 mmol) and stirred for 30 min. To the suspension was transferred bromide compound **13** in CH_2Cl_2 (44 mL) and allowed to stir at room temperature for 24 h. The reaction was quenched with MeOH (10 mL). The mixture was then filtered by Celite pad. The filtrate was concentrated *in vacuo* and purified by flash chromatography on silica gel (EtOAc/hexane, 8:92 to 15:85) to afford yellow syrup as product. (16.7 g, 96% from serine ester **4**). $C_{28}H_{28}BrNO_4$; TLC (EtOAc/hexane, 15:85) R_f= 0.4; diastereomeric ratio: 83:17; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 7.85 (2 H, t, *J* = 8.0 Hz), 7.43–7.35 (4 H, m), 7.32–7.22 (5 H, m), 7.18 (1 H, d, *J* = 7.5 Hz), 4.42 (0.17H, m), 4.32 (0.83H, t, *J* = 3.4 Hz), 3.55–3.51 (3H, m), 3.35–3.30 (2H, m), 3.17 (2.49H, s), 3.15 (0.51H, s), 2.76–2.71 (1 H, m), 1.57–1.28 (6 H, m) ; ^{13}C{^{1}H} (125.77 MHz, DMSO-d_6) δ 173.8/173.6, 148.5/148.4, 148.2/148.1, 144.1, 140.0, 139.9, 131.0, 128.6/128.5, 128.1, 128.0, 127.6, 125.5, 124.7, 120.3, 120.1, 98.0/96.8, 72.0, 68.4/67.7, 61.2/60.5, 55.9/55.6, 51.1/51.0, 29.9/29.7, 24.8, 18.8/18.4; HRMS (ESI) calcd for $C_{28}H_{29}{}^{81}BrNO_4$: 524.1259.; found: *m*/*z* 524.1252 $[M + H]^+$.



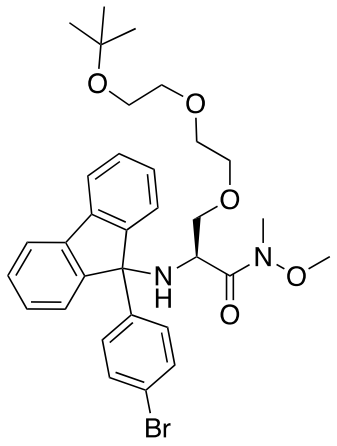
8

*2-N-(9-(4-Bromophenyl)-9-fluorenyl)-3-hydroxyl-N-methoxy-N-methylpropanamide (**8**)*. To a chilled solution of methyl ester **5** (10.0 g, 19.1 mmol) in a co-solvent of ethanol and water (400 mL, 1:1) was added LiOH-H_2O (3.21 g, 76.6 mmol) portion-wise at 0 ^oC in an ice bath. The reaction mixture was allowed to gradually warm to and stir at ambient temperature for 12 h. The mixture was neutralized with Dowex resin and filtered. The filtrate was concentrated *in vacuo* and co-evaporated with toluene (3x) to afford compound 6 as white solid. The crude mixture was used in the next step without further purification.

To the above compound 6 was added anhydrous DMF and CH_2Cl_2 (200 mL, 3:1), followed by DCC (5.93 g, 28.7 mmol) and DhBtOH (4.68 g, 28.7 mmol) at 0 ^oC in an ice bath. After 1 h of stirring, a mixture of *N*,*O*-dimethylhydroxylamine (2.80 g, 28.7 mmol), DIEA (5.00 mL, 28.7 mmol) in anhydrous DMF and CH_2Cl_2 (200 mL, 3:1) was transferred to the above reaction mixture at 0 ^oC. The resulting solution was stirred for 2 h at ambient temperature. The reaction was then quenched with methanol and concentrated *in vacuo*. The resulting syrup was diluted with EtOAc (50 mL). The organic layer was washed with sat. $NaHCO_3$ (3x) and brine (3x), dried over Na_2SO_4, and concentrated *in vacuo* to afford Weinreb amide 7 as yellowish foaming solid. The crude product was used in the next step without further purification.

To the above compound 7 was added methanol (95.7 mL) and *p*-toluenesulfonic acid monohydrate (3.64 g, 19.1 mmol) at 0 ^oC and stirred at ambient temperature for 18 h. The reaction mixture was neutralized with sat. $NaHCO_3$ and concentrated *in vacuo.* The resulting solid was diluted with EtOAc and H_2O. The aqueous layer was washed with EtOAc (2x). The

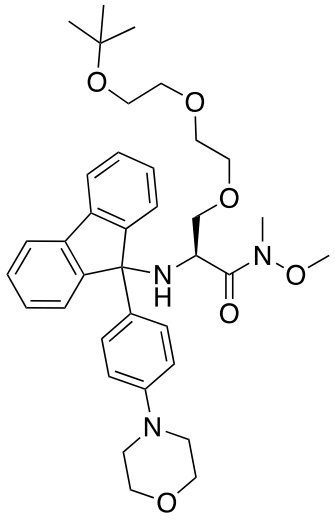
combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was then purified by column chromatography (EtOAc: hexane= 40:60 to 85:15) to afford alcohol **8** (8.50 g, 95% in three steps). Colorless syrup; $C_{24}H_{23}BrN_2O_3$; TLC (EtOAc/hexane, 65:35) R_f= 0.4; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 7.83 (2 H, d, *J* = 8.1 Hz), 7.43–7.35 (5 H, m), 7.30–7.24 (4 H, m), 7.10 (1 H, d, *J* = 7.6 Hz), 4.63 (1H, t, *J* = 5.9 Hz), 3.33–3.24 (3H, m), 2.95 (4H, br), 2.72 (3H, br); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 173.8, 149.0, 148.2, 144.5, 140.5, 139.5, 131.1, 128.4, 128.1, 127.9, 127.5, 125.7, 125.7, 120.1, 72.1, 64.0, 59.9, 53.8, 31.5; HRMS (ESI) calcd for $C_{24}H_{24}BrN_2O_3$: 467.0970; found: *m*/*z* 467.0954 $[M + H]^+$.



9

2-N-(9-(4-Bromophenyl)-9-fluorenyl)-3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-N-methoxy-N-methylpropanamide (**9**). To a chilled solution of alcohol **8** (7.73g, 16.5 mmol eq) in DMF (0.2 M) in an ice bath was added 60% NaH (1.00 g, 25.0 mmol) and stirred at the same temperature. After 30 min, tetrabutylammonium iodide (0.10 g, 0.17 mmol) and tosylate **17** (10.5 g, 19.9 mmol) were added to the above suspension. After 12 h, the reaction was quenched with sat. NH_4Cl. The mixture was concentrated *in vacuo,* followed by stirring with EtOAc and water. The aqueous layer was washed with EtOAc (2x). The combined organic layer was then washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 22:78 to 53:47) to afford colorless syrup (7.80 g,

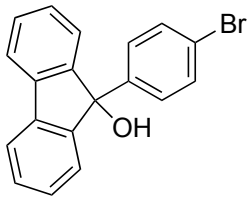
77%). $C_{32}H_{39}BrN_2O_5$; TLC (EtOAc/hexane, 50:50) R_f= 0.4; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 7.83 (2 H, d, *J* = 6.3 Hz), 7.42–7.34 (5 H, m), 7.29–7.23 (4 H, m), 7.10 (1 H, d, *J* = 7.5 Hz), 3.40–3.27 (11H, m), 3.17 (1H, br), 2.99 (3H, br), 2.71 (3 H, br), 1.09 (9 H, s); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 173.5, 148.8, 148.2, 140.4, 139.6, 131.1, 128.5, 128.0, 127.9, 127.5, 125.6, 124.9, 120.2, 73.1, 72.2, 72.0, 70.4, 70.0, 69.6, 60.6, 60.0, 51.4, 31.5, 27.2; HRMS (ESI) calcd for $C_{32}H_{40}{}^{81}BrN_2O_5$: 613.2100; found: *m*/*z* 613.2085 $[M + H]^+$.



10

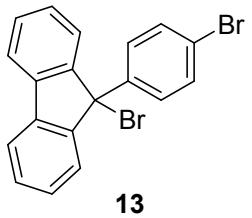
3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-N-methoxy-N-methyl-2-N-(9-(4-morpholinophenyl)-9-fluorenyl)-propanamide (**10**). To a solution of bromide **9** (7.50 g, 12.2 mmol) in degassed toluene (24 mL) was added $Pd(OAc)_2$ (137.7 mg, 0.61 mmol), XPhos (292.31 mg, 0.61 mmol), cesium carbonate (20.0 g, 61.3 mmol) and morpholine (1.29 mL, 14.7 mmol). The resulting mixture was stirred at 80 ^{o}C for 2 h. After gradual cooling to ambient temperature, the reaction mixture was filtered through Celite pad. The filtrate was concentrated *in vacuo* and purified by flash column chromatography on NH_2 silica gel (EtOAc/hexane, 18:72 to 63:37) to afford **10** as colorless syrup (7.27 g, 96%). $C_{36}H_{47}N_3O_6$; TLC (EtOAc/hexane, 50:50) R_f= 0.2; ^{1}H NMR (300.13 MHz, DMSO-d_6) δ 7.82–7.78 (2 H, m), 7.38–7.07 (8 H, m), 6.78 (2H, d, *J* = 9.0 Hz), 3.68 (4H, t, *J* = 4.7 Hz), 3.38–3.22 (11 H, m), 3.11 (1H, br), 3.03–2.97 (7 H, m), 2.72 (3H, br),

1.10 (9H, s); $^{13}C\{^1H\}$ NMR (125.77 MHz, DMSO-d_6) δ 173.7, 150.0, 149.7, 148.8, 140.3, 139.4, 135.0, 128.1, 127.7, 127.2, 126.3, 125.6, 124.9, 120.0, 1144.7, 73.3, 72.2, 72.0, 70.4, 70.0, 69.6, 66.0, 60.6, 59.3, 51.5, 48.3, 31.5, 27.2; HRMS (ESI) calcd for $C_{36}H_{47}N_3NaO_6$: 640.3363; found: *m*/*z* 640.3338 $[M + Na]^+$.



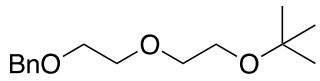
12

9-(4-Bromophenyl)-9-fluorenol (**12**).[45] To a solution of 1,4-dibromobenzene (72.1 g, 305.5 mmol) in 1L of ether at – 50 °C in an ACN-dry ice bath was added *n*-butyllithium (1M, 25 mL) over a period of 1 h and stirred for another 30 min. To this suspension was transferred fluorenone (38.5 g, 213.8 mmol) in 400 mL of ether under N_2 atmosphere and stirred at the same temperature for another 1 h. The reaction mixture was gradually warmed to ambient temperature and stirred for 2 h. The mixture was cooled to 0 °C and quenched with water (200 mL). The aqueous layer was washed with ether (300 mL 2x). The combined organic layer was washed with brine (300mL x 2), dried over $MgSO_4$, and concentrated *in vacuo* to afford the crude product as yellow syrup. The residue was purified by flash column chromatography on silica gel (EtOAc/hexane, 1:49 to 1:9) to afford compound **12** (62.6 g, 87% yield from fluorenone). $C_{19}H_{13}BrO$, white solid; TLC (EtOAc/hexane, 15:85) R_f= 0.4; ^1H NMR (500.13 MHz, $CDCl_3$) δ 7.65 (2 H, d, *J* = 7.8 Hz), 7.38–7.35 (4H, m), 7.28–7.22 (6H, m), 2.41 (1H, br); $^{13}C\{^1H\}$ NMR (125.77 MHz, $CDCl_3$) δ 150.0, 142.3, 139.5, 131.3, 129.3, 128.6, 127.3, 124.7, 121.2, 120.2, 83.3.



13

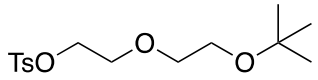
*9-(4-bromophenyl)-9-fluorenyl bromide (**13**)*: To an anhydrous solution of 9-(4-bromophenyl)-9-fluorenol **12**[45] (14.1 g, 41.8 mmol) in toluene (208.0 mL) was added acetyl bromide (61.9 mL, 835.1 mmol) at 0 °C and refluxed at 120 °C for 2 h. Upon the completion of the reaction as confirmed by GC-MS, the reaction mixture was concentrated under reduced pressure and co-evaporated with toluene three more times. The residue was dried *in vacuo* for 12 h to afford the bromide **13** as yellowish syrup. This compound was used in the next step without further purification. For analytical sample: ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 7.66 (2H, d, *J* = 7.7 Hz), 7.47 (2H, d, *J* = 7.4 Hz), 7.41 (2H, d, *J* = 8.6 Hz), 7.37–7.34 (4H, m), 7.29–7.26 (2H, m); ^{13}C{^{1}H} NMR (125.77 MHz, $CDCl_3$) δ 149.1, 140.4, 138.0, 131.4, 129.2 (2x), 128.6, 125.9, 122.2, 120.4, 66.5. GC-MS t_R= 17.8 min.



15

((2-(2-(tert-butoxy)ethoxy)ethoxy)methyl)benzene (**15**). To a chilled solution of ethylene glycol monobenzyl ether (157.0 g, 0.80 mol) in CH_2Cl_2 (1.0 L) at -50 °C in an ACN/dry ice bath was added Boc anhydride (401.6 g, 1.84 mol) and $Mg(ClO_4)_2$ (17.9 g, 0.80 mol). The reaction mixture was allowed to gradually warm to room temperature and refluxed for 36 h. The reaction was quenched by adding sat. $NaHCO_3$ (200 mL). The aqueous layer was washed with CH_2Cl_2 (200 mL 2x). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography on silica gel (EtOAc/hexane, 1:99 to 16:84) to afford compound **15** (144.3 g, 71%). $C_{15}H_{24}NO_3$;

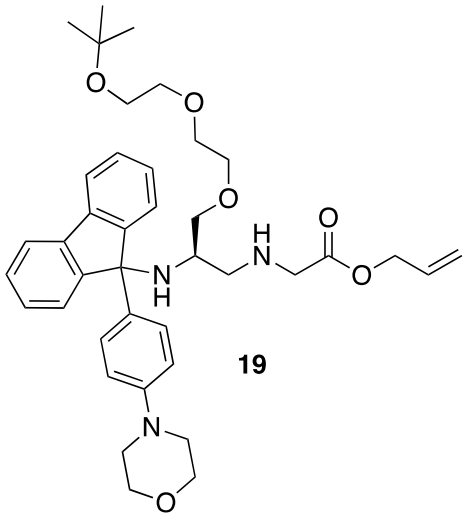
colorless syrup; TLC (EtOAc/hexane, 15:85) R_f= 0.6; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 7.35–7.26 (5 H, m), 4.57 (2 H, s), 3.70–3.68 (2 H, m), 3.64–3.60 (4H, m), 3.53 (2H, t, *J*= 5.4 Hz), 1.20 (9H, s); ^{13}C{^{1}H} NMR (125.77 MHz, $CDCl_3$) δ 138.3, 128.3, 127.7, 127.5, 73.2, 73.0, 71.2, 70.7, 69.4, 61.1, 27.5; HRMS (ESI) calcd for $C_{15}H_{24}NaO_3$: 275.1623; found: *m*/*z* 275.1606 [M + Na]$^+$.



17

2-(2-(tert-butoxy)ethoxy)ethyl-4-methylbenzenesulfonate (**17**). To a chilled solution of *tert*-butyl ether **15** (144.3 g, 0.57 mol) in MeOH (50 mL) at 0 °C was added Pd/C (608.5 mg, 0.57 mmol). The reaction was stirred at 100 psi of H_2 gas for 12 h at ambient temperature. The reaction mixture was then cooled to 0 °C and filtered through Celite. The filtrate was concentrated *in vacuo* to afford alcohol 16, which was used in the next step without purification.

To a chilled solution of alcohol 16 in CH_2Cl_2 (1.0 L) at 0 °C was added DMAP (41.9 g, 0.34 mol), Et_3N (79.7 mL, 9.57 mol) and tosyl chloride (130.8 g, 0.69 mol). The reaction mixture was stirred at ambient temperature for 12 h before quenching with 10% citric acid (200 mL). The organic layer was further washed with 10% citric acid (200 mL) and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography on silica gel (EtOAc/hexane, 1:99 to 35:65) to afford tosylate **17** (163.1 g, 90% in two steps) as colorless syrup. $C_{15}H_{24}O_5S$; colorless syrup; TLC (EtOAc/hexane, 22:78) R_f= 0.4; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 7.80 (2H, t, *J* = 8.2 Hz), 7.34 (2H, t, *J* = 8.2 Hz), 4.16 (2H, t, *J* = 9.8 Hz), 3.70 (2H, t, *J* = 9.8 Hz), 3.53 (2H, t, *J* = 10.3 Hz), 3.44 (2H, t, *J* = 10.3 Hz) 2.44 (3H, s), 1.17 (9H, s); ^{13}C{^{1}H} NMR (125.77 MHz, $CDCl_3$) δ 144.7, 133.1, 129.8, 128.0, 73.0, 71.4, 69.3, 68.7, 61.2, 27.4, 21.6; HRMS (ESI) calcd for $C_{15}H_{24}NaO_5S$: 339.1242; found: *m*/*z* 339.1228 [M + Na]$^+$.



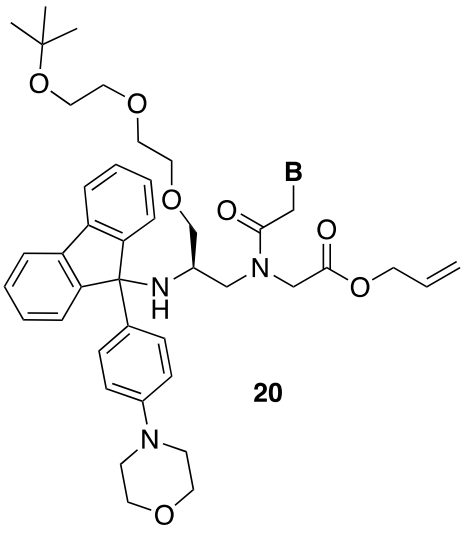
19

3-(2-(2-(tert-butoxy)ethoxy)ethoxy-2-N-(9-(4-morpholinophenyl)-9-fluorenyl))-serine-Ψ [CH_2N]Gly-O-allyl (**19**). To a chilled solution of amide **10** (423.9 mg, 0.69 mmol) in anhydrous THF (7 mL) at 0 °C was added lithium aluminum hydride (26.0 mg, 0.69 mmol). The reaction mixture was stirred for 1 h before quenching with sat. Na_2SO_4 and further diluted with ether. The aqueous layer was then extracted with ether (2x). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to afford aldehyde 18. The residue was used in the next step without purification.

To a chilled solution of aldehyde 18 in anhydrous dichloroethane (7 mL) at 0 °C was added glycine allyl ester toluenesulfonic acid salt[55] (197.2 mg, 0.69 mmol), *N*,*N*-diisopropylethylamie (239.0 µL, 1.37 mmol) and sodium triacetoxyborohydride (218.1 mg, 1.03 mmol). The reaction mixture was stirred at ambient temperature for 20 h before quenching with sat. $NaHCO_3$. The mixture was then diluted with ether. The aqueous layer was extracted with ether (2x). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/hexane, 24:76 to 99:1) to afford backbone **19** as colorless syrup (370.1 mg, 82% in two steps). $C_{39}H_{51}N_3O_6$; TLC (EtOAc/hexane, 80:20) R_f= 0.4; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 7.81–7.79 (2 H, m), 7.37–7.20 (6 H, m), 7.17 (2H, d, *J* = 9.0 Hz), 6.77 (2H, d, *J* = 9.0 Hz), 5.95–5.87 (1 H, m), 5.30 (1H,

dq, *J* = 17.3, 1.6 Hz), 5.22 (1H, dq, *J* = 10.5, 1.4 Hz), 4.56 (2H, d, *J* = 4.8 Hz), 3.68 (4H, t, *J* = 4.8 Hz), 3.37–3.30 (7 H, m), 3.13 (2H, t, *J* = 4.8 Hz), 3.09 (1H, d, *J* = 17.2 Hz), 3.03–2.92 (7 H, m), 2.26 (1H, dd, *J* = 11.4, 5.3 Hz), 2.16–2.08 (2 H, m), 2.02 (1H, br), 1.08 (9H, s); $^{13}C\{^{1}H\}$ NMR (125.77 MHz, DMSO-d_6) δ 171.8, 150.6, 150.1, 150.0, 139.7(2x), 136.1, 132.6, 128.0(2x), 127.5(2x), 126.4, 125.3, 125.0, 120.0(2x), 117.8, 114.7, 72.2, 72.0(2x), 70.4, 69.5, 69.4, 66.0, 64.3, 60.6, 51.4, 50.8, 50.2, 48.4, 27.3; HRMS (ESI) calcd for $C_{39}H_{52}N_3O_6$: 658.3856; found: *m*/*z* 658.3826 $[M + H]^+$.

A general procedure for nucleobase coupling



20

To a solution of amine (1.0 eq) in anhydrous DMF (0.1 M) was added *N*,*N*-diisopropylethylamine (2.0 eq), nucleobase (2.0 eq) and HBTU (1.8 eq). The reaction was stirred for 12 h before quenching with MeOH. The reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude was purified by flash column chromatography on NH_2 silica gel (MeOH/EtOAc, 1:99 to 15:85).

3-(2-(2-(Tert-butoxy)ethoxy)ethoxy)-2-N-(9-(4-morpholinophenyl)-9-fluorenyl)-L-serine thymine allyl ester (**20a**). This compound was prepared using the aforementioned general procedure

starting with amine **19** (62.5 mg) and thymine acetic acid (34.99 mg). Yield: (75 mg, 96%); $C_{46}H_{57}N_5O_9$; colorless syrup; TLC (MeOH/EtOAc, 5:95) R_f= 0.5 with NH_2 Silica gel; rotamer ratio=4:1; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 11.27–11.22 (1H, m), 7.84–7.78 (2H, m), 7.44–7.11 (9H, m), 6.79–6.75 (2H, m), 5.97–5.79 (1H, m), 5.37–5.20 (2H, m), 4.60–4.36 (4H, m), 3.69–3.67 (4H, m), 3.60 (1H, d, *J*= 17.1 Hz), 3.49–3.23 (9H, m), 3.15–2.74 (9H, m), 2.21–2.07 (1H, m), 1.74–1.71 (3H, m), 1.07–1.06 (9H, m); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 168.7/168.2, 167.8/167.4, 164.4/164.3, 150.9/150.8, 150.6/150.3, 150.0/149.9, 149.8/149.5, 142.1/141.9, 140.1/139.9, 139.6/139.5, 135.6/135.2, 132.2, 128.3, 128.2/128.1, 127.8, 127.7/127.6, 126.6/126.4, 125.5/125.4, 125.3/125.2, 120.2, 120.1/120.0, 118.4/118.0, 114.8, 108.2/108.0, 72.2(2x), 71.2, 70.4/70.3, 69.9/69.6, 69.5/69.4, 66.0, 65.5/64.8, 60.6/60.5, 51.0/50.6, 50.5/50.3, 48.4, 47.8/47.6, 47.5, 27.3/27.2, 11.9; HRMS (ESI) calcd for $C_{46}H_{58}N_5O_9$: 824.4234; found: 824.4204 *m*/*z* $[M + H]^+$.

3-(2-(2-(Tert-butoxy)ethoxy)ethoxy)-2-N-(9-(4-morpholinophenyl)-9-fluorenyl)-L-serine adenine(Boc) allyl ester (**20b**). This compound was prepared using the aforementioned general procedure starting with amine **19** (71.1 mg) and adenine (Boc) acetic acid (63.40 mg). Yield: (70 mg, 70%); $C_{51}H_{64}N_8O_9$; colorless syrup; TLC (MeOH/$CHCl_3$, 5:95) R_f= 0.4; rotamer ratio=1:3; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 10.05–10.02 (1H, m), 8.50–8.47 (1H, m), 8.22–8.20 (1H, m), 7.83–7.73 (2H, m), 7.48–7.07 (8H, m), 6.80–6.69 (2H, m), 6.01–5.78 (1H, m), 5.40–5.10 (4.75H, m), 4.66–4.46 (2H, m), 4.17 (0.25H, m), 3.69–3.68 (6H, m), 3.43–2.75 (16H, m), 2.20 (1H, br), 1.48 (9H, s), 1.07 (2.25H, s), 1.01 (6.75H, s); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 168.8/168.2, 167.3/166.8, 152.2, 151.6/151.3, 151.4/151.1, 150.6/150.3, 150.1/149.9, 149.8/149.6, 149.7/149.5, 144.8, 140.2/139.9, 139.6/139.5, 135.6/135.2, 132.2/132.1, 128.3, 128.1, 127.8, 127.7/127.6, 126.6/126.4, 125.5/125.4, 125.3, 123.0/122.9, 120.2, 120.1, 118.4/118.0, 114.8/114.7, 80.1/80.0, 72.3/72.2, 72.2/71.9, 71.1, 70.4/70.3, 70.1/69.7, 69.5/69.4,

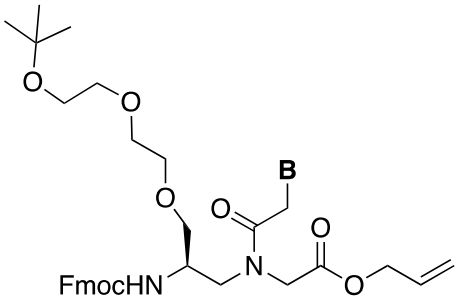
66.0, 65.6/64.8, 60.6/60.4, 51.0/50.7, 50.3, 48.4, 47.6, 44.0/43.5, 27.9, 27.2; HRMS (ESI) calcd for $C_{51}H_{65}N_8O_9$: 933.4874; found: *m*/*z* 933.4847 $[M + H]^+$.

3-(2-(2-(Tert-butoxy)ethoxy)ethoxy)-2-N-(9-(4-morpholinophenyl)-9-fluorenyl)-L-serine cytosine(Boc) allyl ester (**20c**). This compound was prepared using the aforementioned general procedure starting with amine **19** (74.8 mg) and cytosine (Boc) acetic acid (61.23 mg). Yield: (85.4 mg, 83%); $C_{50}H_{64}N_6O_{10}$; colorless syrup; TLC (MeOH/$CHCl_3$, 15:85) R_f= 0.5; rotamer ratio=1:4; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 10.26–10.23 (1H, m), 7.83–7.71 (3H, m), 7.45–7.18 (8H, m), 7.12–6.75 (4H, m), 5.98–5.50 (1H, m), 5.37–5.20 (2H, m), 4.71–4.46 (4.8H, m), 4.05–4.01 (0.2H, m), 3.69–3.67 (4.2H, m), 3.58 (0.8H, d, *J*= 17.1 Hz), 3.47–2.75 (16H, m), 2.23–2.08 (1H, m), 1.46–1.45 (9H, m), 1.07–1.05 (9H, m); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 168.8/168.2, 167.9/167.3, 163.2, 154.9, 152.1, 150.6/150.4, 150.2/150.1, 149.9/149.7, 149.5, 140.1/140.0, 139.6/139.5, 135.6/135.2, 132.2, 128.2, 128.1, 127.8, 127.7, 126.6/126.4, 125.4, 125.2, 120.2/120.0, 120.1/119.9, 118.3/118.0, 114.8/114.7, 93.9/93.8, 80.9, 72.3, 72.2, 71.1, 70.4/70.3, 69.9/69.6, 69.5/69.4, 66.0, 65.5/64.8, 60.6/60.4, 51.0/50.7, 50.5/50.2, 49.4/48.8, 48.4, 47.6, 27.8, 27.3/27.2; HRMS (ESI) calcd for $C_{50}H_{64}N_6NaO_{10}$: 931.4552; found: *m*/*z* 931.4542 $[M + Na]^+$.

3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-2-N-(9-(4-morpholinophenyl)-9-fluorenyl)-L-serine guanine(Boc) allyl ester (**20d**). This compound was prepared using the aforementioned general procedure starting with amine **19** (71.1 mg) and guanine (Boc) acetic acid (66.85 mg). Yield: (76.8 mg, 75%); $C_{51}H_{64}N_8O_{10}$; colorless syrup; TLC (MeOH/$CHCl_3$, 10:90) R_f= 0.2 with NH2 Silica gel; rotamer ratio=1:3; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 11.3 (1H, br), 11.0–10.9 (1H, br), 7.84–7.77 (2H, m), 7.69–7.67 (1H, m), 7.47–7.08 (8H, m), 6.80–6.73 (2H, m), 6.01–5.80 (1H, m), 5.40–5.20 (2H, m), 4.89–4.80 (2.5H, m), 4.64–4.63 (0.5H, m), 4.48–4.47 (1.5H, m), 4.20–4.02 (0.5H, m), 3.70–3.68 (4H, m), 3.54–2.76 (18H, m), 2.22–2.12 (1H, m), 1.49–1.48 (9H, m), 1.07 (2.25H, s), 1.01 (6.75H, s); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 168.9/168.1,

167.2/166.7, 155.1, 153.6, 150.5/150.2, 150.1/149.9, 149.7/149.4, 149.5/149.4, 147.3, 140.2, 140.1/139.9, 139.7/139.5, 135.6/135.1, 132.2, 128.3, 128.2, 128.1, 127.9, 127.7, 126.5/126.3, 125.4, 125.3, 125.2, 120.2/120.1, 119.0/118.9, 114.8/114.7, 82.5/82.4, 72.3/72.2, 72.2/72.0, 71.0, 70.4/70.2, 70.0/69.7, 69.5/69.4, 66.0, 65.6/64.9, 60.6/60.3, 51.1/50.6, 50.4, 48.4, 47.5, 43.7/43.4, 27.8, 27.3/27.1; HRMS (ESI) calcd for $C_{51}H_{65}N_8O_{10}$: 949.4823; found: *m*/*z* 949.4807 $[M + H]^+$.

A general method for converting MPhF- to Fmoc-protected allyl ester 22a-d



22

To a chilled solution of MPhF-protected allyl ester **20** (1 eq) in ACN (0.1M) at 0 °C was added 1M citric acid (3.6 eq). The reaction mixture was allowed to gradually warm to and stir at ambient temperature for 12 h. The reaction progress was monitored by HRMS (See Figure S28 and S29). Upon completion, the reaction mixture was chilled in an ice bath for 10 min, followed by the addition of $NaHCO_3$ (14 eq) and Fmoc-Cl (2.5 eq). After 3 h, the reaction mixture was mixed with silica gel and concentrated *in vacuo* and directly purified by column chromatography (EtOH/$CHCl_3$= 1:99 to 10:90) to afford the final Fmoc-MPγPNA allyl ester **22**.

Fmoc-3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-L-serine thymine monomer allyl ester (**22a**). This compound was prepared using the aforementioned general procedure starting with ally ester **20a** (59.50 mg). Yield: (45 mg, 86%); $C_{38}H_{48}N_4NaO_{10}$; colorless syrup; TLC (MeOH/$CHCl_3$, 5:95) R_f= 0.5; rotamer ratio= 2:3; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 11.27–11.26 (1H, m), 7.89 (2H,

d, *J*= 7.5 Hz), 7.70 (2H, d, *J*= 7.4 Hz), 7.43–7.22 (5H, m), 5.99–5.85 (1H, m), 5.40–5.19 (2H, m), 4.74–4.04 (10H, m), 3.90–3.80 (1H, m), 3.59–3.08 (12H, m), 1.73–1.72 (3H, m), 1.09–1.08 (9H, m); $^{13}C\{^{1}H\}$ NMR (125.77 MHz, DMSO-d_6) δ 169.0/168.5, 168.0/167.7, 164.3, 155.8/155.7, 150.9, 143.9/143.8, 142.0/141.9, 140.7, 132.2, 127.6, 127.0, 125.2/125.1, 120.1, 118.2/117.8, 108.2/108.0, 72.2, 70.5, 70.4, 69.9/69.8, 69.6/69.5, 65.5, 64.8, 60.6/60.5, 49.9/49.3, 48.4, 48.2/48.0, 47.8/47.7, 46.7, 27.3/27.2, 11.9; HRMS (ESI) calcd for c 743.3268; found: *m*/*z* 743.3244 $[M + Na]^+$.

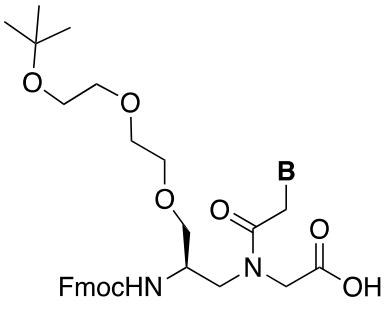
Fmoc-3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-L-serine adenine(Boc) monomer allyl ester (**22b**). This compound was prepared using the aforementioned general procedure starting with allyl ester **20b** (43.50 mg). Yield: (33 mg, 85%); $C_{43}H_{55}N_7O_{10}$; colorless syrup; TLC (MeOH/$CHCl_3$, 5:95) R_f= 0.5; rotamer ratio= 2:3; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 10.06 (1H, br), 8.55–8.53 (1H, m), 8.34–8.29 (1H, m), 7.92–7.90 (2H, m), 7.74–7.71 (2H, m), 7.53–7.32 (4H, m), 6.00–5.85 (1H, m), 5.46–5.19 (4H, m), 4.73–3.15 (21H, m), 1.51 (9H, m), 1.11 (9H, m); $^{13}C\{^{1}H\}$ NMR (125.77 MHz, DMSO-d_6) δ 169.0/168.4, 167.4/167.1, 155.9/155.8, 152.2, 151.4, 151.1, 149.7, 144.8, 143.9/143.8, 140.7, 132.2, 127.6, 127.0, 125.2, 123.0/122.9, 120.1, 118.2/117.8, 80.0/79.1, 72.2, 70.5, 70.4, 70.1/69.8, 69.6, 65.6/65.5, 64.8, 60.6, 50.2/49.9, 48.5, 48.2, 46.7, 27.8, 27.3/27.2; HRMS (ESI) calcd for $C_{43}H_{56}N_7O_{10}$: 830.4088; found: *m*/*z* 830.4069 $[M + H]^+$.

Fmoc-3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-L-serine cytosine(Boc) monomer allyl ester (**22c**). This compound was prepared using the aforementioned general procedure starting with allyl ester **20c** (59.80 mg). Yield: (48 mg, 91%); $C_{42}H_{55}N_5O_{11}$; colorless syrup; TLC (MeOH/$CHCl_3$, 5:95) R_f= 0.3; rotamer ratio=1:1; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 10.27 (1H, br), 7.89–6.96 (10H, m), 5.98–5.84 (1H, m), 5.37–5.18 (2H, m), 4.88–4.79 (1H, m), 4.65–4.55 (3H, m), 4.41–4.21 (4H, m), 4.15–4.04 (1H, m), 3.91–3.80 (1H, m), 3.57–3.10 (13H, m), 1.45 (9H, s), 1.09–1.08 (9H, m); $^{13}C\{^{1}H\}$ NMR (125.77 MHz, DMSO-d_6) δ 169.0/168.5, 168.0/167.7, 163.3, 155.9/155.7, 155.0, 152.1, 150.4, 143.9/143.8, 140.7, 132.3/132.2, 127.6, 127.0, 125.1, 120.8, 118.2/117.8,

93.9, 80.9, 72.2, 70.4(2x), 69.9/69.8, 69.6/69.5, 65.5/65.4, 64.8, 60.6/60.5, 50.0, 49.5/49.4, 49.3, 48.3/48.2, 46.7, 27.8, 27.3/27.2; HRMS (ESI) calcd for $C_{42}H_{56}N_5O_{11}$: 806.3976; found: *m*/*z* 806.3930 $[M + H]^+$.

Fmoc-3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-L-serine guanine(Boc) monomer allyl ester (**22d**). This compound was prepared using the aforementioned general procedure starting with allyl ester **20d** (53.20 mg) as the starting material. Yield: (39 mg, 82%); $C_{43}H_{55}N_7O_{11}$; colorless syrup; TLC (MeOH/$CHCl_3$, 5:95) R_f= 0.1; rotamer ratio=1:1; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 11.31–10.69 (2H, br), 7.89–7.22 (9H, m), 6.01–5.83 (1H, m), 5.37–5.19 (2H, m), 5.18–3.35 (23H, m), 1.45–1.39 (9H, m), 1.08–1.05 (9H, m); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 169.1/168.5, 167.4/167.1, 156.1/155.8, 155.1/155.0, 153.7/153.6, 149.6/149.4, 147.4, 143.8, 143.6, 140.7, 140.3/140.1, 132.2/132.1, 127.6, 127.5/127.3, 127.0, 125.1/125.0, 121.3, 120.2/120.1, 118.9, 118.3/117.8, 82.5/82.4, 72.2, 70.6, 70.4, 70.0/69.9, 69.8/69.6, 65.6/65.5, 65.4/64.9, 60.6/60.5, 50.5, 49.6/49.4, 48.4/48.2, 46.7, 43.8/43.6, 27.7/27.6, 27.2; HRMS (ESI) calcd for $C_{43}H_{56}N_7O_{11}$: 846.4038; found: *m*/*z* 846.3983 $[M + H]^+$.

A general method for converting Fmoc-protected allyl esters 22a-d to the final monomers 1a-d



1

To a solution of allyl ester **22** (1 eq) in DCM (0.1M) was added $Pd[PPh_3]_4$ (0.2 eq) and $PhSiH_3$ (2 eq). The reaction was stirred at ambient temperature for 12 h. The reaction mixture was then concentrated *in vacuo* with silica gel. The crude was then purified by column chromatography

(A= EtOAc, B= ACN/MeOH/H_2O= 2:1:1, A:B= 97:3 to 66:34) to afford the final Fmoc-MPγPNA monomers **1**.

Fmoc-3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-L-serine thymine monomer (**1a**). 96% yield; $C_{35}H_{44}N_4O_{10}$; white powder; TLC (EtOAc/ACN/MeOH/H_2O, 6:1:1:1) R_f= 0.5; rotamer ratio=2:3; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 12.9 (1 H, br), 11.3–11.2 (1 H, m), 7.88 (2 H, *J*= 7.6 Hz, d), 7.69 (2 H, *J*= 7.4 Hz, d), 7.43–7.22 (5 H, m), 4.72–4.64 (1 H, m), 4.46 (1 H, s), 4.35–4.28 (2 H, m), 4.23–4.22 (1 H, m), 4.11 (1 H, m), 4.02–3.80 (2 H, m), 3.60–3.07 (13 H, m), 1.72 (3 H, m), 1.09–1.08 (9 H, m); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 170.9/170.4, 168.0/167.5, 164.4, 155.9/155.8, 151.0/150.9, 143.9/143.8, 142.1/142.0, 140.7, 127.6, 127.0, 125.2, 120.1, 108.1/108.0, 72.2, 70.5, 70.4, 69.9/69.8, 69.6, 65.6/65.4, 60.6/60.5, 49.8/49.4, 48.5, 48.1/47.7, 47.8, 46.7, 27.3/27.2, 11.9; HRMS (ESI) calcd for $C_{35}H_{44}N_4NaO_{10}$: 703.2955; found: *m*/*z* 703.2927 $[M + H]^+$.

Fmoc-3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-L-serine adenine(Boc) monomer (**1b**). 90% yield; $C_{40}H_{51}N_7O_{10}$; white powder; TLC (EtOAc/ACN/MeOH/H_2O, 6:1:1:1) R_f= 0.4; rotamer ratio=2:3; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 12.8 (1 H, br), 10.0 (1 H, s), 8.51–8.50 (1 H, m), 8.27 (1 H, s), 7.88 (2 H, *J*= 7.3 Hz, d), 7.72–7.69 (2 H, m), 7.51–7.14 (4 H, m), 5.42–5.10 (2 H, m), 4.38–4.21 (4 H, m), 4.06–3.81 (2 H, m), 3.70–3.12 (13 H, m), 1.48 (9 H, m), 1.08 (3.6 H, s), 1.04 (5.4 H, s); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 170.7/170.1, 167.4/166.9, 155.9/155.8, 152.2, 151.4, 151.1, 149.7, 144.9/144.8, 143.9/143.8, 140.7, 128.9/127.6, 128.2/127.0, 125.3/125.2, 122.9, 120.1, 80.0, 72.2, 70.5, 70.4, 70.0, 69.7, 69.6, 65.6/65.4, 60.6/60.5, 49.8/49.3, 48.5, 48.0, 46.7 , 27.8, 27.2; HRMS (ESI) calcd for $C_{40}H_{52}N_7O_{10}$: 790.3775; found: *m*/*z* 790.3749 $[M + H]^+$.

Fmoc-3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-L-serine cytosine(Boc) monomer (**1c**). 92% yield; $C_{39}H_{51}N_5O_{11}$; white powder; TLC (EtOAc/ACN/MeOH/H_2O, 6:1:1:1) R_f= 0.5; rotamer ratio=1:1; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 10.26 (1 H, br), 7.88 (2 H, *J*= 7.6 Hz, d), 7.84–7.79 (1 H,

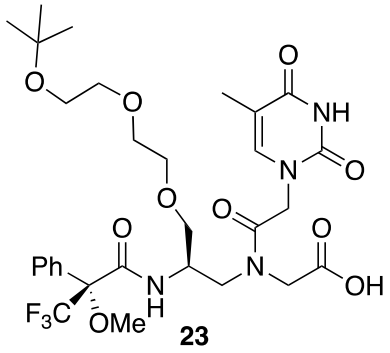
m), 7.71–7.68 (2 H, m), 7.44–7.40 (2 H, m), 7.34–7.27 (2 H, m), 6.67–6.95 (1 H, m), 4.87–4.77 (1 H, m), 4.62 (1 H, s), 4.33–4.20 (3 H, m), 4.03–3.79 (3 H, m), 3.58–3.00 (14 H, m), 1.45 (9 H, s), 1.09–1.08 (9 H, m); $^{13}C\{^{1}H\}$ NMR (125.77 MHz, DMSO-d_6) δ 171.2, 167.9/167.3, 163.2, 155.9/155.8, 155.1/155.0, 152.1, 150.5, 143.9/143.8, 140.7, 127.6, 127.0, 125.2, 120.1, 94.0/93.9, 80.9, 72.2, 70.5, 70.4, 69.9/69.8, 69.6, 65.5/65.4, 60.6/60.5, 51.1/49.8, 49.6/49.5, 49.4/48.5, 48.3/47.9, 46.7, 27.8, 27.3/27.2; HRMS (ESI) calcd for $C_{39}H_{52}N_5O_{11}$: 766.3663; found: *m*/*z* 766.3643 $[M + H]^+$.

Fmoc-3-(2-(2-(tert-butoxy)ethoxy)ethoxy)-L-serine guanine(Boc) monomer (**1d**). 88% yield; $C_{40}H_{51}N_7O_{11}$; white powder; TLC (EtOAc/ACN/MeOH/H_2O, 6:1:1:1) R_f= 0.3; rotamer ratio=1:1; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 11.3–10.7 (2 H, br), 7.88–7.60 (5 H, m), 7.42–7.21 (4 H, m), 5.15–4.84 (2 H, m), 4.37–3.08 (20 H, m), 1.44–1.38 (9 H, m), 1.07–1.05 (9 H, m); $^{13}C\{^{1}H\}$ NMR (125.77 MHz, DMSO-d_6) δ 171.1/170.6, 167.4/166.7, 156.1/155.8, 155.1/155.0, 153.8/153.6, 149.6/149.5, 147.5/147.4, 143.8, 143.6, 140.7, 140.3, 127.6, 127.5/127.3, 127.0, 125.2/125.0, 121.3, 120.2/120.1, 118.9, 82.5/82.4, 72.2, 70.6, 70.4, 70.0/69.8, 69.7, 65.6/65.4, 60.6/60.5, 51.4/50.3, 49.5/48.8, 48.6/47.9, 46.8/46.7, 43.9/43.8, 27.7/27.6, 27.2; HRMS (ESI) calcd for $C_{40}H_{52}N_7O_{11}$: 806.3725; found: *m*/*z* 806.3693 $[M + H]^+$.

Determination of optical purities

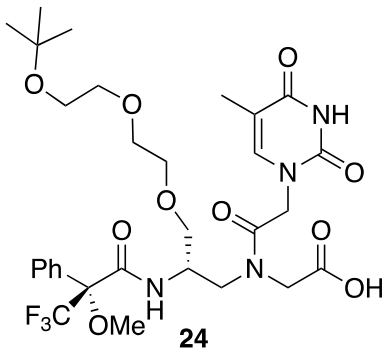
To a stirred, cold solution of thymine monomers **1a** and **2a** (1.0 eq) in separate flasks in DMF was added piperidine (10 eq). After 2 h, the reaction mixture was concentrated and co-evaporated with toluene three times. The residue was then stirred in chilled CH_2Cl_2, followed by addition of DIEA (2 eq) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (MTPACl; 1.1 eq). The reaction mixture was stirred at room temperature for 3 h. Upon completion, the solution was diluted with CH_2Cl_2 and washed with water (2x) and brine. The organic layer was dried on Na_2SO_4 and concentrated *in vacuo.* The crude sample was directly

injected into HPLC. For NMR analysis, the crude sample was purified by flash column chromatography.



23

Compound **23**: $C_{30}H_{41}F_3N_4O_{10}$; colorless syrup TLC (EtOAc/ACN/MeOH/H_2O, 6:1:1:1) R_f= 0.5; rotamer ratio=1:1; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 12.9 (1 H, br), 11.3 (1 H, m), 8.27–8.14 (1 H, m), 7.55–7.41 (5 H, m), 7.13–7.07 (1 H, m), 4.61–4.27 (3 H, m), 3.94–3.11 (17 H, m), 1.74–1.72 (3 H, m), 1.11 (9 H, m); ^{13}C{^{1}H} NMR (125.77 MHz, DMSO-d_6) δ 170.5/ 170.2, 168.1/167.5, 165.8/165.5, 164.4/164.3, 158.4/158.1, 150.9, 141.9, 133.4/133.2, 129.4, 128.4, 127.0, 127.2:124.9:122.6:120.3 (1:3:3:1, $\underline{C}F_3$, $J_{C\text{-}F}$ = 289 Hz), 108.1/108.0, 83.7/83.5, 72.3, 70.4, 70.0/69.7, 69.7/69.3, 60.6, 55.0/54.9, 49.2, 47.8/47.7, 47.7/47.6, 47.4/47.2, 27.3/27.2, 11.9/11.8); ^{19}F NMR (470.56 MHz, DMSO) δ -70.0/-70.1; HRMS (ESI) calcd for $C_{30}H_{41}F_3N_4NaO_{10}$: 697.2672; found: *m*/*z* 697.2649 [M + Na]$^+$.



24

Compound **24**: $C_{30}H_{41}F_3N_4O_{10}$; colorless syrup TLC (EtOAc/ACN/MeOH/H_2O, 6:1:1:1) R_f= 0.5; rotamer ratio=1:1; ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 13.1 (1 H, br), 11.3 (1 H, m), 8.35–8.05

(1 H, m), 7.53–7.42 (5 H, m), 7.27–7.22 (1 H, m), 4.76–4.63 (1 H, m), 4.48 (1 H, s), 4.35–4.20 (2 H, m), 4.03–3.92 (1 H, m), 3.65–3.34 (15 H, m), 1.75 (3 H, m), 1.11–1.10 (9 H, m); $^{13}C\{^{1}H\}$ NMR (125.77 MHz , DMSO-d_6) (CF_3 quartet merged with aromatic carbons) δ 171.1/170.7, 168.9/168.0, 166.2/166.0, 164.9/164.8, 158.5, 151.4, 142.5/142.3, 133.6/133.2, 130.0, 129.8, 128.9, 128.7, 128.0, 127.8, 127.5, 108.7/108.6, 72.7, 70.9, 70.8, 70.2, 70.1, 69.5, 61.0, 55.5/55.4, 49.6, 48.3, 48.2, 48.0, 47.9, 47.4, 27.7, 12.3; ^{19}F NMR (470.56 MHz, DMSO) δ -70.1; HRMS (ESI) calcd for $C_{30}H_{41}F_3N_4NaO_{10}$: 697.2672; found: *m*/*z* 697.2643 $[M + Na]^+$.

ASSOCIATED CONTENTS

Supporting Information

Synthesis schemes and HPLC, NMR, and HR-MS spectra of the compounds made.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Khvorova, A.; Watts, J. K. The chemical evolution of oligonucleotide therapies of clinical utility. *Nat. Biotech.* **2017**, *35*, 238-248.

(2) Rinaldi, C.; Wood, M. J. A. Antisense oligonucleotides: the next frontier for treatment of neurological disorders. *Nat. Rev. Neurol.* **2018**, *14*, 9-21.

(3) Jones, M. R.; Seeman, N. C.; Mirkin, C. A. Programmable materials and the nature of the DNA bond. *Science* **2015**, *347*, 1260901.

(4) Adleman, L. M. Molecular computation of solutions to combinatorial problems. *Science* **1994**, *266*, 1021-1024.

(5) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science* **1991**, *254*, 1497-1500.

(6) Nielsen, P. E. Peptide nucleic acid. A molecule with two identities. *Acc. Chem. Res.* **1999**, *32*, 624-630.

(7) Masuko, M. Hybridization of an immobilized PNA probe with its complementary oligodeoxyribonucleotide on the surface of silica glass. *Nucleic Acids Res. Suppl.* **2003**, 145-146.

(8) Cattani-Scholz, A.; Pedone, D.; Blobner, F.; Abstreiter, G.; Schwartz, J.; Tornow, M.; Andruzzi, L. PNA-PEG modified silicon platforms as functional bio-interfaces for applications in DNA microarrays and biosensors. *Biomacromolecules* **2009**, *10*, 489-496.

(9) Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. Peptide nucleic acids (PNA). Oligonucleotide analogues with an achiral peptide backbone. *J. Am. Chem. Soc.* **1992**, *114*, 1895-1897.

(10) Haaima, G.; Lohse, A.; Buchardt, O.; Nielsen, P. E. Peptide nucleic acids (PNAs) containing thymine monomers derived from chiral amino acids: Hybridization and solubility properties of D-lysine PNA. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1939-1941.

(11) Sforza, S.; Tedeschi, T.; Corradini, R.; Marchelli, R. Induction of helical handedness and DNA binding properties of peptide nucleic acids (PNAs) with two stereogenic centres. *Eur. J. Org. Chem.* **2007**, 5879-5885.

(12) Dose, C.; Seitz, O. Convergent synthesis of peptide nucleic acids by native chemical ligation. *Org. Lett.* **2005**, *7*, 4365-4368.

(13) Englund, E. A.; Appella, D. H. Synthesis of gamma-substituted peptide nucleic acids: a new place to attach fluorophores without affecting DNA binding. *Org. Lett.* **2005**, *7*, 3465-3467.

(14) Tedeschi, T.; Sforza, S.; Corradini, R.; Marchelli, R. Synthesis of new chiral PNAs bearing a dipeptide-mimic monomer with two lysine-derived stereogenic centres. *Tetrahedron Lett.* **2005**, *46*, 8395-8399.

(15) Mitra, R.; Ganesh, K. N. Aminomethylene peptide nucleic acid (am-PNA): Synthesis, regio-/stereospecific DNA binding, and differential cell uptake of (alpha/gamma, R/S)am-PNA analogues. *J. Org. Chem.* **2012**, *77*, 5696-5704.

(16) Avitabile, C.; Moggio, L.; Malgieri, G.; Capasso, D.; Di. Gaetano, S.; Saviano, M.; Pedone, C.; Romanelli, A. Gamma sulphate PNA (PNA S): Highly selective DNA binding molecule showing promising antigene activity. *PLoS ONE* **2012**, *7*, 1-10.

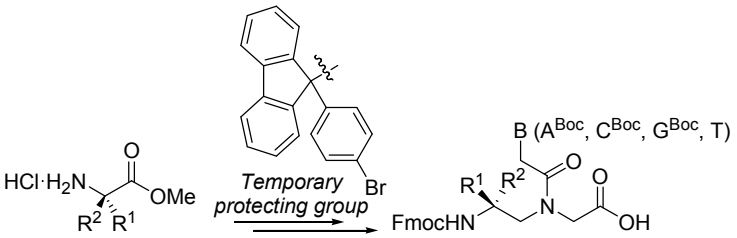
(17) De Costa, N. T. S.; Heemstra, J. M. Evaluating the effect of ionic strength on duplex stability for PNA having negatively or positively charged side chains. *PLoS ONE* **2013**, *8*, 1-8.

(18) Chang, D.; Lindberg, E.; Winssinger, N. Critical analysis of rate constants and turnover frequency in nucleic acid-templated reactions: reaching terminal velocity. *J. Am. Chem. Soc.* **2017**, *139*, 1444-1447.
(19) Roloff, A.; Seitz, O. Reducing product inhibition in nucleic acid-templated ligation reactions: DNA-templated cyclization. *ChemBioChem* **2013**, *14*, 2322-2328.
(20) Bandlow, V.; Liese, S.; Lauster, D.; Ludwig, K.; Netz, R. R.; Herrmann, A.; Seitz, O. Spatial screening of hemagglutinin on influenza A virus particles: Sialyl-LacNAc displays on DNA and PEG scaffolds reveal the requirements for bivalency enhanced interactions with weak monovalent binders. *J. Am. Chem. Soc.* **2017**, *139*, 16389-16397.
(21) Dragulescu-Andrasi, A.; Rapireddy, S.; Frezza, B. M.; Gayathri, C.; Gil, R. R.; Ly, D. H. A simple gamma-backbone modification preorganizes peptide nucleic acid into a helical structure. *J. Am. Chem. Soc.* **2006**, *128*, 10258-10267.
(22) Sacui, I.; Hsieh, W.-C.; Manna, A.; Sahu, B.; Ly, D. H. Gamma peptide nucleic acids: As orthogonal nucleic acid recognition codes for organizing molecular self-assembly. *J. Am. Chem. Soc.* **2015**, *137*, 8603-8610.
(23) Bahal, R.; Sahu, B.; Rapireddy, S.; Lee, C.-M.; Ly, D. H. Sequence-unrestricted, Watson-Crick recognition of double helical B-DNA by (R)-MiniPEG-gPNAs. *ChemBioChem* **2012**, *13*, 56-60.
(24) Zaghloul, E. M.; Madsen, A.; Moreno, P. M. D.; Oprea, I. I.; El-Andaloussi, S.; Bestas, B.; Gupta, P.; Pedersen, E. B.; Lundin, K. E.; Wengel, J.; Smith, C. I. E. Optimizing anti-gene oligonucleotide 'Zorro-LNA' for improved strand invasion into duplex DNA. *Nucleic Acid Res.* **2011**, *39*, 1142-1154.
(25) Sahu, B.; Sacui, I.; Rapireddy, S.; Zanotti, K. J.; Bahal, R.; Armitage, B. A.; Ly, D. H. Synthesis and characterization of conformationally preorganized, (R)-diethylene glycol-containing g-peptide nucleic acids with superior hybridization properties and water solubility. *J. Org. Chem.* **2011**, *76*, 5614-5627.
(26) Singer, A.; Rapireddy, S.; Ly, D. H.; Meller, A. Electronic barcoding of a viral gene at the single-molecule level. *Nano Lett.* **2012**, *12*, 1722-1728.
(27) Nolling, J.; Rapireddy, S.; Amburg, J. I.; Crawford, E. M.; Prakash, R. A.; Rabson, A. R.; Tang, Y.-W.; Singer, A. Duplex DNA-invading y-modified peptide nucleic acids enable rapid identification of bloodstream infections in whole blood. *MBio* **2016**, *7*, e00345-16.
(28) Morin, T. J.; Shropshire, T.; Liu, X.; Briggs, K.; Huynh, C.; Tabard-Cossa, V.; Wang, H.; Dunbar, W. B. Nanopore-based target sequence detection. *PLOS One* **2016**, 0154426.
(29) Bahal, R.; McNeer, N. A.; Quijano, E.; Liu, Y.; Sulkowski, P.; Bhunia, D. C.; Manna, A.; Greiner, D. L.; Brehm, M. A.; Cheng, C. J.; Lopez-Giraldez, F.; Beloor, J.; Krause, D. S.; Kumar, P.; Gallagher, P. G.; Braddock, D.; Saltzman, W. M.; Ly, D. H.; Glazer, P. M. In vivo correction of anemia in b-thalassemic mice by yPNA-mediated gene editing with nanoparticle delivery. *Nat. Commun.* **2016**, *7*, 13304.
(30) Ricciadi, A. S.; Bahal, R.; Farrelly, J. S.; Quijano, E.; Bianchi, A. H.; Luks, V. L.; Putman, R.; Lopez-Giraldez, F.; Coskun, S.; Song, E.; Liu, Y.; Hsieh, W.-C.; Ly, D. H.; Stitelman, D. H.; Glazer, P. M.; Saltzman, W. M. In utero nanoparticle delivery for site-specific genome editing. *Nat. Commun.* **2018**, 2481.
(31) Kosynkina, L.; Wang, W.; Liang, T. C. A convenient synthesis of chiral peptide nucleic acid (PNA) monomers. *Tetrahedron Lett.* **1994**, *35*, 5173-5176.
(32) Rittle, K. E.; Homnick, C. F.; Ponticello, G. S.; Evans, B. E. A synthesis of statine utilizing an oxidative route to chiral alpha-amino aldehydes. *J. Org. Chem.* **1982**, *47*, 3016-3018.
(33) Stanfield, C. F.; Parker, J. E.; Kanellis, P. Preparation of protected amino aldehydes. *J. Org. Chem.* **1981**, *46*, 4797-4798.
(34) Miles, N. J.; Sammes, P. G.; Kennewell, P. D.; Westwood, R. On the double-bond isostere of the peptide-bond-preparation of modified di-peptide and tri-peptide

incorporating proline and alanine analogs. *J. Chem. Soc. Perkin. Trans. 1* **1985**, 2299-2305.
(35) Dellaria, J. F. J.; Maki, R. G. The enantio- and diastereoselective synthesis of the first phospho-statine derivative. *Tetrahedron Lett.* **1986**, *27*, 2337-2340.
(36) Barlos, K.; Papaioannou, D.; Theodoropoulos, P. Efficient "one-pot" synthesis of N-trityl amino acids. *J. Org. Chem.* **1982**, *47*, 1324-1326.
(37) Chouikhi, D.; Ciobanu, M.; Zambaldo, C.; Duplan, V.; Barluenga, S.; Winssinger, N. 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. *Chem.-Eur. J.* **2012**, *18*, 12698-12704.
(38) Myers, A. G.; Zhong, B.; Movassaghi, M.; Kung, D. W.; Lanman, B. A.; Kwon, S. Synthesis of highly epimerizable N-protected alpha-amino aldehydes of high enantiomeric excess. *Tetrahedron Lett.* **2000**, *41*, 1359-1362.
(39) Reetz, M. T. Synthesis and diastereoselective reactions of N,N-dibenzylamino aldehydes and related compounds. *Chem. Rev.* **1999**, *99*, 1121-1162.
(40) Lubell, W. D.; Rapoport, H. Surrogates for chiral aminomalondialdehyde. Synthesis of N-(9-phenylfluoren-9-yl)serinal and N-(-9-phenylfluoren-9-yl)vinylglycinal. *J. Am. Chem. Soc.* **1989**, *54*, 3824-3831.
(41) Gosselin, F.; Betsbrugge, J. V.; Hatam, M.; Lubell, W. D. A novel linking-protecting group strategy for solid-phase organic chemistry with configurationally stable alpha[N-(Phenylfluorenyl)]amino carbonyl compounds: Synthesis of enantiopure norephedrines on solid support. *J. Org. Chem.* **1999**, *64*, 2486-2493.
(42) Lubell, W. D.; Rapoport, H. Configurational stability of N-protected alpha-amino aldehydes. *J. Am. Chem. Soc.* **1987**, *109*, 236-239.
(43) Manna, A.; Rapireddy, S.; Sureshkumar, G.; Ly, D. H. Synthesis of optically-pure yPNA monomers: a comparative study. *Tetrahedron* **2015**, *71*, 3507-3514.
(44) Pothukanuri, S.; Pianowski, Z.; Winssinger, N. Expanding the scope and orthogonality of PNA synthesis. *Eur. J. Org. Chem.* **2008**, 3141-3148.
(45) Surprenant, S.; Lubell, W. D. 9-(4-Bromophenyl)-9-fluorenyl as a safety-catch nitrogen protecting group. *J. Org. Chem.* **2006**, *71*, 848-851.
(46) Asahina, Y.; Takei, M.; Kimura, T.; Fukuda, Y. Synthesis and antibacterial activity of novel pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid derivatives carrying the 3-cyclopropylaminomethyl-4-substituted-1-pyrrolidinyl group as a C-10 substituent. *J. Med. Chem.* **2008**, *51*, 3238-3249.
(47) Shimizu, T.; Osako, K.; Nakata, T. Efficient method for preparation of N-methyl-N-methyl amides by reaction of lactones or esters with Me2AlCl-MeONHMe*HCl. *Tetrahedron Lett.* **1997**, *38*, 2685-2688.
(48) Beaudoin, D.; Grenon, O. L.; Maris, T.; Wuest, J. D. Building giant carbocycles by reversible C-C bond formation. *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 894-898.
(49) Christie, B. D.; Rapoport, H. Synthesis of optically pure pipecolates from L-asparagine - application to the total synthesis of (+)-Apovincamine through amino acid decarboxylation and iminium ion cyclization. *J. Org. Chem.* **1985**, *50*, 1239-1246.
(50) Bartoli, G.; Bosco, M.; Lacatelli, M.; Marcantoni, E.; Melchiorre, P.; Sambri, L. Unusual and unexpected reactivity of t-butyl dicarbonate (Boc2) with alcohols in the presence of magnesium perchlorate: a new route to t-butyl ethers. *Org. Lett.* **2005**, *7*, 427-430.
(51) Wright, S. W.; Hageman, D. L.; Wright, A. S.; McClure, L. D. Convenient preparations of t-butyl esters and ethers from t-butanol. *Tetrahedron Lett.* **1997**, *38*, 7345-7348.
(52) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. Reductive amination of aldehydes and ketones with sodium triacetoxyborohydride. *J. Org. Chem.* **1996**, *61*, 3849-3862.

(53) Heuer-Jungemann, A.; Howarth, N. M.; Ja'Afaru, S. C.; Rosair, G. M. Development of a convenient route for the preparation of the N2-Cbz-protected guaninyl synthon required for Boc-mediated PNA synthesis. *Tet. Lett.* **2013**, *54*, 6275-6278.
(54) Seco, J. M.; Quinoa, E.; Riguera, R. The assignment of absolute configuration by NMR. *Chem. Rev.* **2004**, *104*, 17-117.
(55) Xia, Z. P.; Smith, C. D. Efficient synthesis of a fluorescent farnesylated Ras peptide. *J. Org. Chem.* **2001**, *66*, 5241-5244.

TOC



SM1 (*L*): R^1= CH_2OH, R^2= H
SM2 (*D*): R^1= H, R^2= CH_2OH

1a-d *are monomers for preparing the right-handed oligos, and* ***2a-d*** *are for preparing the left-handed version.*

1a-d: from **SM1**
R^1= $CH_2(OCH_2CH_2)_2O^tBu$, R^2= H
2a-d: from **SM2**
R^1= H, R^2= $CH_2(OCH_2CH_2)_2O^tBu$
High optical purity (>99.5% ee)