Synthesis of 2-Aminopyridine-Modified Peptide Nucleic Acids

Ilze Kumpina [a,b]
Vladislavs Baskevics[b]
Grant D. Walby[c]
Brandon R. Tessier[a]
Sara Farshineh Saei[a]
Christopher A. Ryan[a]
James A. MacKay [c]
Martins Katkevics [b]
Eriks Rozners*[a]

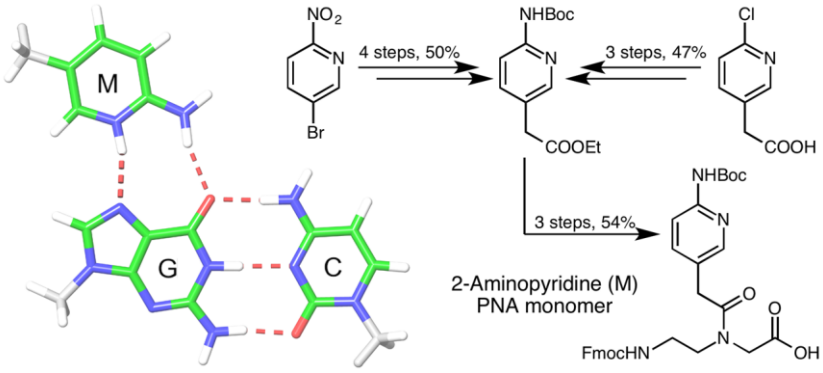
[a] Department of Chemistry, Binghamton University, The State University of New York, Binghamton, New York 13902, United States.

[b] Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga, LV-1006, Latvia.

[c] Department of Chemistry and Biochemistry, Elizabethtown College, Elizabethtown, Pennsylvania 17022, United States.

erozners@binghamton.edu

Click here to insert a dedication.



Received:
Accepted:
Published online:
DOI:

Abstract Triplex-forming peptide nucleic acids (PNAs) require chemical modifications for efficient, sequence specific recognition of DNA and RNA at physiological pH. Our research groups have developed 2-aminopyridine (M) as an effective mimic of protonated cytosine in C+•G-C triplets. M-modified PNAs have high binding affinity and sequence specificity, and promising biological properties for improving PNA applications. This communication reports optimization of synthetic procedures that give PNA M monomer in seven steps, with minimal need for column chromatography, and in good yields and purity. The optimized route uses inexpensive reagents and easy to perform reactions, which will be useful for the broad community of nucleic acid chemists. Thought has also been given to potential for future development of industrial syntheses of M monomers.

Key words PNA, modified nucleobases, RNA recognition, triple helix, PNA monomer synthesis, solid phase PNA synthesis.

Peptide nucleic acid (PNA, Figure 1) was introduced by Nielsen and co-workers in 1991 as a ligand for triple-helical binding to double-stranded DNA (dsDNA).[1] PNA binds to both single and double stranded nucleic acids with high affinity and sequence specificity, which has led to many applications of PNAs as research probes and diagnostics.[2] However, the therapeutic potential of PNA has not yet been realized leaving significant room for improvement of PNA's properties by chemical modifications.

An early PNA modification was the replacement of cytosine with pseudoisocytosine (J, Figure 1) to address the problem of unfavorable cytosine protonation (pK_a ~4.5) required for formation of the Hoogsteen C+•G-C triplet in PNA-dsDNA complexes.[3] Chen and co-workers recently improved the binding properties of J by substituting oxygen with sulfur in thio-pseudoisocytosine (L, Figure 1).[4] In 2012, we adopted the more basic 2-aminopyridine (M, pK_a ~6.7), previously used in DNA triplexes,[5] as a superior alternative to J in PNA.[6] Later detailed biophysical studies showed that an M+•G-C triplet had about three times stronger stability than a J•G-C triplet.[7] Moreover, M did not form strong Watson-Crick base pairs with the native nucleobases, which reduced the potential for off-target binding to single-stranded DNA and RNA.[7] PNA-RNA triplexes formed by M-modified PNA have already been used to catalyze template-directed reactions,[8] inhibit mRNA translation[9] and microRNA maturation,[10] and detect A to I editing in RNA.[11] Over the years, we have been developing and optimizing synthesis of PNA monomers and oligomers having M and other nucleobases.[6, 12] In the present manuscript, we summarize these efforts and report the latest optimized synthetic route to Fmoc protected M monomers and M-modified PNAs along with specific advantages and disadvantages of alternative routes.

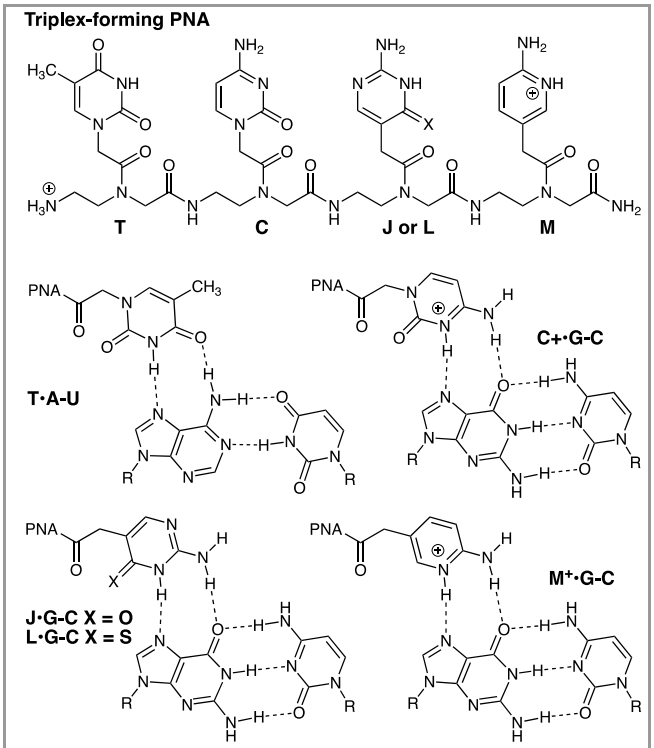
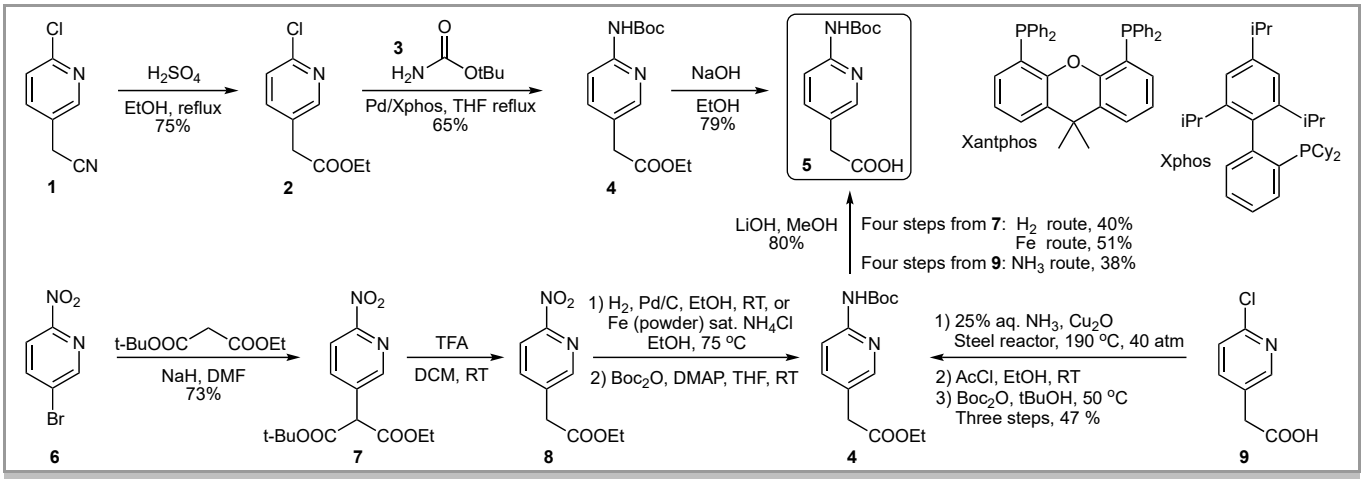


Figure 1 Structures of triplex-forming PNA and Hoogsteen triplets formed by native and modified nucleobases.



Scheme 1 Alternative syntheses of M-acetic acid key intermediate **5**.

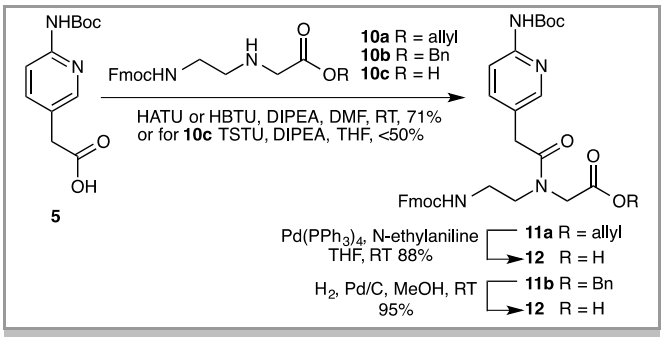
Typically, synthesis of PNA is performed on an automated peptide synthesizer using Fmoc or Boc protected monomers. Some DNA synthesizers, such as the Expedite 8909, may have PNA protocols. We prefer the Fmoc strategy because the milder deprotection conditions allow for the use of Boc-protected nucleobases in custom monomers, such as M. The key step in synthesis of custom Fmoc protected monomers is preparation of the modified heterocycle as an acetic acid derivative (i.e., **5** in Scheme 1) that can be attached to appropriately protected PNA backbone.

Our original synthesis[13] of M monomer followed procedures described in a patent by Burns et al. [14] The synthesis started with ethanolysis of the commercially available 2-(6-chloro-3-pyridinyl) acetonitrile **1** followed by Buchwald-Hartwig amination catalyzed by Xantphos-palladium complex (Scheme 1). However, we found that it was difficult to purify the Boc-protected ester **4** from residual Xantphos and excess carbamate **3**. After some optimization, we discovered that using Xphos-palladium catalyst[15] and 1.1 equivalent of carbamate **3** in refluxing deoxygenated THF gave pure **4** in acceptable yield after silica gel column chromatography (for experimental details, see Supporting Information).

After ester hydrolysis, the optimized original route gave the key acetic acid intermediate **5** in three steps and in a sufficient ~39% overall yield but was costly (**1** and Xphos are expensive) and required an intricate Buchwald-Hartwig amination step, which was difficult to control on larger scales. To further improve the synthesis of M monomer, we developed an alternative route starting from 5-bromo-2-nitropyridine **6** (Scheme 1) that avoids expensive reagents and was easier to scale up. Nucleophilic aromatic substitution with tert-butyl ethyl malonate gave **7** after silica gel column purification.[16, 17] Decarboxylation under acidic conditions was followed by reduction of the nitro group using Fe powder (for details of Fe reduction, see Supporting Information).[16] Most recently, we found that hydrogenation over Pd on carbon was an even simpler and higher yielding alternative to Fe powder reduction.[18] After Boc protection, both routes converged on ester **4**.[19] Providing that good quality reagents and solvents are used under careful control of reaction conditions, the entire sequence (**7** to **4**) required only one silica gel column purification of ester **4**. We have also found that that the aromatic substitution may give sufficiently pure **7** to run the entire synthesis (**6** to **4**) without chromatography purification of any intermediate. Finally, cleavage of the ethyl ester (using either NaOH[13] or LiOH) completed the synthesis of the key acetic acid derivative **5**.[20] In our hands, N-Boc protection has been convenient and effective; however, others have reported PNA synthesis using N-trityl protected M monomers.[21] Overall, the new synthesis gives the key intermediate **5** in five steps and ~37% overall yield. Although it was two steps longer and slightly lower yielding, the new route used inexpensive starting materials and reactions were amenable to scale up and industrial development, though some reagents (e.g., NaH and trifluoroacetic acid) may need to be replaced in industrial settings.

To explore even more efficient alternatives, we developed a one-pot three-step sequence to **4** starting from chloropyridine **9**. The amino group was introduced by heating **9** in aqueous ammonia at 190 °C (warning, a steel reactor required due to pressure reaching 40 atm!) in the presence of copper (I) oxide. The main side reaction is formation of ~25% of 2-(6-hydroxypyridine-3-yl)acetic acid. Nevertheless, during the extraction and crystallization procedures at subsequent esterification and Boc protection steps, this and other minor impurities were removed and **4** was obtained with 98% (HPLC) purity and 47% yield over the three steps (for experimental details, see Supporting Information). No chromatography was used in this procedure; however, it is less attractive for laboratory use due to the necessity of high-pressure equipment.

In our original procedure,[13] we coupled **5** with O-allyl protected PNA backbone **10a** (Scheme 2). During synthesis of various modified PNAs,[12, 16] we discovered that using O-benzyl protected backbone **10b** with either HATU or HBTU as coupling reagents provided a simpler and cleaner route to PNA monomers. In our optimized route to **12** (Scheme 2), we recommend using the O-benzyl protected backbone **10b**; however, other heterocyclic PNA bases (e.g., L and others containing sulfur) may not be compatible with hydrogenation conditions. In such cases, the O-allyl protected backbone **10a** may provide a better alternative. In cases when the nucleobase is not compatible with either Pd catalyst, a free acid **10c** can be used with TSTU as a coupling reagent under anhydrous conditions, albeit the yields are usually lower (<50%).[16]



Scheme 2 Coupling to PNA backbones and final steps towards M-monomer.

While **10a** and **10b** are stable when stored as solids at -20 °C, both degrade rapidly in solution (estimated $t_{1/2}$ ~4 days) and as solids (estimated $t_{1/2}$ ~2-3 weeks) at room temperature. The salt forms of **10a** and **10b** are more stable, but generally, salts of either **5** or **10a** and **10b** give lower yields in the coupling reactions. Hydrogenation over Pd on carbon gives pure (no column chromatography needed) final M monomer **12** that is stable in NMP solution (used for PNA synthesis on Expedite) at -20 °C for more than a month.[22]

Synthesis of modified PNAs can be performed using either an Expedite 8909 DNA synthesizer that has Fmoc-PNA protocol or peptide synthesizers. We have previously described and are routinely using Expedite to synthesize M-modified PNAs on a 2 μmol scale.[6, 7, 23] However, alternative approaches are needed because manufacturing of Expedites has been discontinued for more than two decades and the number of available refurbished instruments is declining. Among peptide synthesizers, the microwave assisted CEM Liberty instruments have been a convenient alternative, especially for synthesis of PNA-peptide conjugates.[24, 25] Using the previous reports as a starting point,[24, 25] we briefly optimized the synthesis of M-modified PNAs on a CEM Liberty Blue instrument on a 10 μmol scale (for details, see Supporting Information). Consistent with previous observations,[24] coupling at 40 °C for 20 min gave higher yields than protocols that used 50 °C (c.f., Figures S3 and S4). Overall, we obtained good amounts and satisfactory yields (Table S1) of pure 9-mer PNAs similar to those used in our recent studies on PNA-RNA triplexes.[26]

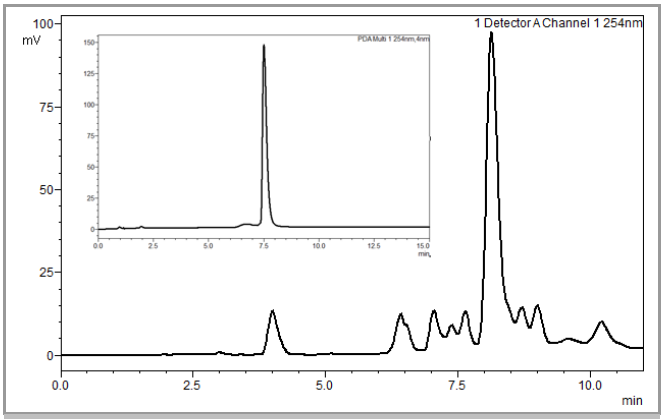


Figure 2 HPLC profiles of crude and purified (inset) PNA NH_2-TMTMMTMMT-$CONH_2$ synthesized on CEM Liberty Blue using M monomer **12** (for details of LC conditions, see Figure S3).

In summary, we have developed a seven-step synthetic route that gives PNA M monomer **12** in ~27% overall yield. Overall, the yields may depend on the scale of reactions with smaller scale preparations giving higher yields than those we report and, vice versa, larger scales may somewhat decrease the yields. Despite minimal use of column chromatography (only two or three over seven steps) the route gives **12** with sufficient purity for multistep solid phase synthesis PNA oligomers. The purity of **12** (and other PNA monomers) is critical as in our hands contaminations that are sometimes not detectable by NMR or LCMS (e.g., salts, etc.) lead to failures of PNA syntheses. In such cases, it is possible to do additional column purification of the final monomer to restore the coupling efficiency. Our optimized route to **12** uses straightforward reactions that should be easy to adopt for academic labs and industrial synthesis.

Funding Information

This work was supported by the National Science Foundation (CHE-1708761 and CHE-2107900 to E.R., and CHE-1708699 and CHE-2107911 to J.A.M.). I.K. and V.B. were supported by the Latvian Institute of Organic Synthesis internal research fund (IG-2019-04 and IG-2023-08).

Acknowledgment

We thank Tristan Mabee for screening conditions for the direct sequence of **6** to **4**.

Supporting Information

YES

Primary Data

NO.

Conflict of Interest

The authors declare no conflict of interest.

References and Notes

(1) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497.
(2) (a) Brodyagin, N.; Katkevics, M.; Kotikam, V.; Ryan, C. A.; Rozners, E. *Beilstein J. Org. Chem.* **2021**, *17*, 1641. (b) Saarbach, J.; Sabale, P. M.; Winssinger, N. *Curr. Opin. Chem. Biol.* **2019**, *52*, 112.
(3) Egholm, M.; Christensen, L.; Dueholm, K. L.; Buchardt, O.; Coull, J.; Nielsen, P. E. *Nucleic Acids Res.* **1995**, *23*, 217.
(4) (a) Devi, G.; Yuan, Z.; Lu, Y.; Zhao, Y.; Chen, G. *Nucleic Acids Res.* **2014**, *42*, 4008. (b) Hildbrand, S.; Blaser, A.; Parel, S. P.; Leumann, C. J. *J. Am. Chem. Soc.* **1997**, *119*, 5499. (c) Bates, P. J.; Laughton, C. A.; Jenkins, T. C.; Capaldi, D. C.; Roselt, P. D.; Reese, C. B.; Neidle, S. *Nucleic Acids Res.* **1996**, *24*, 4176. (d) Cassidy, S. A.; Slickers, P.; Trent, J. O.; Capaldi, D. C.; Roselt, P. D.; Reese, C. B.; Neidle, S.; Fox, K. R. *Nucleic Acids Res.* **1997**, *25*, 4891.
(5) Devi, G.; Yuan, Z.; Lu, Y.; Zhao, Y.; Chen, G. *Nucleic Acids Res.* **2014**, *42*, 4008.
(6) (a) Zengeya, T.; Gupta, P.; Rozners, E. *Angew. Chem., Int. Ed.* **2012**, *51*, 12593. (b) Hnedzko, D.; McGee, D. W.; Karamitas, Y. A.; Rozners, E. *RNA* **2017**, *23*, 58.
(7) Ryan, C. A.; Brodyagin, N.; Lok, J.; Rozners, E. *Biochemistry* **2021**, *60*, 1919.
(8) Kim, K. T.; Chang, D.; Winssinger, N. *Helv. Chim. Acta* **2018**, *101*, e1700295.
(9) Endoh, T.; Hnedzko, D.; Rozners, E.; Sugimoto, N. *Angew. Chem., Int. Ed.* **2016**, *55*, 899.

(10) Endoh, T.; Brodyagin, N.; Hnedzko, D.; Sugimoto, N.; Rozners, E. *ACS Chem. Biol.* **2021**, *16*, 1147.

(11) Annoni, C.; Endoh, T.; Hnedzko, D.; Rozners, E.; Sugimoto, N. *Chem. Commun.* **2016**, *52*, 7935.

(12) (a) Kumpina, I.; Baskevics, V.; Nguyen, K. D.; Katkevics, M.; Rozners, E. *ChemBioChem* **2023**, *24*, e202300291. (b) Brodyagin, N.; Maryniak, A. L.; Kumpina, I.; Talbott, J. M.; Katkevics, M.; Rozners, E.; MacKay, J. A. *Chem. - Eur. J.* **2021**, *27*, 4332.

(13) Hnedzko, D.; Cheruiyot, S. K.; Rozners, E. *Curr. Protoc. Nucleic Acid Chem.* **2014**, *58*, 4.60.1.

(14) Burns, C. J.; Goswami, R.; Jackson, R. W.; Lessen, T.; Li, W.; Pevear, D.; Tirunahari, P. K.; Xu, Patent 2010-EP56408 2010130708, **2010**.

(15) (a) Qin, L.; Cui, H.; Zou, D.; Li, J.; Wu, Y.; Zhu, Z.; Wu, Y. *Tetrahedron Lett.* **2010**, *51*, 4445. (b) Surry, D. S.; Buchwald, S. L. *Chem. Sci.* **2011**, *2*, 27. (c) Bhagwanth, S.; Waterson, A. G.; Adjabeng, G. M.; Hornberger, K. R. *J. Org. Chem.* **2009**, *74*, 4634.

(16) Kumpina, I.; Brodyagin, N.; MacKay, J. A.; Kennedy, S. D.; Katkevics, M.; Rozners, E. *J. Org. Chem.* **2019**, *84*, 13276.

(17) **1-(tert-Butyl)-3-ethyl-2-(6-nitropyridin-3-yl)malonate** (**7**). *tert*-Butyl ethyl malonate (31.7 mL, 167 mmol, 2 equiv) was added dropwise to a suspension of NaH (60% dispersion in mineral oil, 6.70 g, 167 mmol, 2 equiv) in dry DMF (100 mL) at 0 °C (ice bath) and under an argon atmosphere. The suspension was stirred at room temperature till the mixture become a clear solution, then 5-bromo-2-nitropyridine (**6**) (17.0 g, 83.7 mmol) was added. The reaction mixture was stirred at 80 °C (oil bath) for 20 h, cooled to room temperature, and saturated aqueous NH_4Cl (100 mL) was added. The pH was adjusted to 4 with of aqueous 1 N HCl solution, and the aqueous phase was extracted with EtOAc (4 × 70 mL). The combined organic layers were washed with sat. NaCl (70 mL), dried with Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using a linear gradient of EtOAc (10–25%) in hexanes to afford the title compound **7** (19.0 g, 73% yield) as a light-yellow oil. The characterization data matched the previously reported.[16]

(18) **Ethyl 2-(6-aminopyridin-3-yl)acetate**. Trifluoroacetic acid (TFA) (47.0 mL, 610 mmol, 10 equiv) was added to a solution of 1-(*tert*-butyl)-3-ethyl-2-(6-nitropyridin-3-yl)malonate (**7**) (19.0 g, 61.1 mmol) in CH_2Cl_2 (200 mL) at 0 °C (ice bath). After 23 h at room temperature, the solvent was evaporated. The residue was co-evaporated under reduced pressure with toluene (2 × 50 mL) to afford **8** (15.1 g) that was dissolved in in EtOH (160 mL). N_2 gas was bubbled through this solution for 10 min, then Pd/C (1.60 g, 10% w/w) was added, and reaction mixture was bubbled with H_2 gas. After30 h N_2 gas was bubbled through this solution for 10 min. The mixture was filtered through pad of Celite and washed with EtOH (3 × 15 mL). The combined filtrates were evaporated under reduced pressure to afford the title compound (10.3 g, estimated 93% yield over 2 steps) as a dark yellow oil that was used in the next step without purification.

(19) **Ethyl 2-(6-((tert-butoxycarbonyl)amino)pyridin-3-yl)acetate** (**4**) Boc_2O (6.28 g, 28.8 mmol, 1.5 equiv) and DMAP (0.24 g, 1.92 mmol, 0.1 equiv) were added to the solution of ethyl 2-(6-aminopyridin-3-yl)acetate (3.46 g, 19.2 mmol) in dry THF (50 ml). After 15 h at room temperature, solvent was partly evaporated and the oily residue was purified by silica gel column chromatography using a linear gradient of EtOAc (0–30%) in hexanes to afford the title compound **4** (4.07 g, 68% yield) as a white solid. The characterization data matched the previously reported.[13]

(20) **2-(6-((tert-butoxycarbonyl)amino)pyridin-3-yl)acetic acid** (**5**) A 1M aqueous solution of LiOH (49 mL, 48.7 mmol, 2 equiv) was added to solution of **4** (6.83 g, 24.4 mmol) in EtOH (60 mL). After 6 h at room temperature solvent was partly evaporated and residue was acidified with 1M HCl solution in water to pH ~ 5. Formed precipitates were filtered and washed with cold water (3 × 10 mL) to afford the title compound **5** (4.89 g, 80% yield) as a white solid. The characterization data matched the previously reported.[13]

(21) Tähtinen, V.; Granqvist, L.; Murtola, M.; Strömberg, R.; Virta, P. *Chem. Eur. J.* **2017**, *23*, 7113.

(22) **M-monomer** (**12**). 2-(6-((*tert*-Butoxycarbonyl)amino)pyridin-3-yl)acetic acid (**5**) (3.26 g, 12.9 mmol, 1 equiv), O-benzyl-protected PNA backbone **10b** (5.56 g, 12.9 mmol), and HBTU (4.90 g, 12.9 mmol) were dissolved in anhydrous DMF (50 mL). The solution was cooled on ice, and diisopropylethyl amine (DIPEA) (5.6 mL, 32.3 mmol, 2.5 equiv) was added. The ice bath was removed, and the solution was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure, the residue was dissolved in EtOAc (70 mL) and extracted with saturated aqueous $NaHCO_3$ (2 × 20 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using a linear gradient of EtOAc (50–100%) in hexanes to afford the benzyl ester intermediate **11b** (6.14 g, 71% yield) as a slightly yellow oil. R_f = 0.44 (Hexane/EtOAc = 3:7). HRMS (ESI/Q-TOF) m/z: [M + H]+ calcd for $C_{38}H_{41}N_4O_7$ 665.2970; found 665.2988. 1H NMR (400 MHz, DMSO-d_6, ppm, mixture of rotamers) δ: 9.74 (1H, s), 8.10 (0.6H, d, *J* = 2.0 Hz), 8.03 (0.4H, d, *J* = 2.0 Hz), 7.91 – 7.85 (2H, m), 7.77 – 7.61 (3H, m), 7.55 – 7.28 (11H, m), 5.16 (0.6 H, s), 5.12 (1.4H, s), 4.45 – 4.27 (2.6H, m), 4.22 (1H, t, *J* = 5.9 Hz), 4.11 (1.4H, s), 3.70 (1.4H, s), 3.56 (0.6H, s), 3.48 (1.4H, t, *J* = 6.5 Hz), 3.39 (0.6H, t, *J* = 6.5 Hz, 1H), 3.23 (1.4H, q, *J* = 6.4 Hz), 3.15 (0.6H, q, *J* = 6.4 Hz), 1.47 (9H, s). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 171.0, 170.7, 169.7, 169.2, 156.4, 156.1, 152.7, 151.0, 151.0, 150.9, 148.1, 148.0, 143.9, 143.8, 140.8, 140.7, 138.9, 138.7, 135.8, 135.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 127.6, 127.0, 125.5, 125.5, 125.1, 125.0, 120.1, 120.1, 111.8, 111.8, 82.5, 79.5, 66.5, 65.8, 65.4, 65.4, 50.1, 48.0, 47.8, 46.7, 35.6, 35.1, 28.0, 27.4. Solution of intermediate **11b** (6.14 g, 9.2 mmol) in MeOH (70 mL) was bubbled with N_2 gas for 10 min, then Pd/C (600 mg) was added and hydrogen gas (1 atm) was bubbled through the reaction mixture. After 1 h N_2 gas was bubbled through this solution for 10 min. The mixture was filtered through a pad of Celite, and the pad was washed with MeOH (4 × 15 mL). The combined filtrates were evaporated under reduced pressure to afford the M monomer **12** (5.0 g, 95% yield) as a white foam. HRMS (ESI/Q-TOF) m/z: [M + H]+ calcd for $C_{31}H_{35}N_4O_7$ 575.2500; found 575.2524. ^1H NMR (400 MHz, DMSO-d_6, ppm, mixture of rotamers) δ 12.7 (1H, br s), 9.78 (1H, s), 8.1 (0.7H, d, *J* = 2.4 Hz), 8.06 (0.3H, d, *J* = 2.4 Hz), 7.88 (2H, d, *J* = 7.5 Hz), 7.74 (1H, d, *J* = 8.6 Hz), 7.68 (2H, d, *J* = 7.5 Hz), 7.59 – 7.52 (1H, m), 7.50 – 7.21 (5H, m), 4.48 – 4.15 (3.7H, m), 3.98 (1.3H, s), 3.70 (1.3H, s), 3.56 (0.7H, s), 3.45 (1.3H, t, *J* = 6.6 Hz), 3.37 (0.7H, t, *J* = 6.6 Hz), 3.23 (1.3H, q, *J* = 6.3 Hz), 3.15 (0.7H, q, *J* = 6.3 Hz), 1.46 (s, 9H). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO) δ 206.5, 171.3, 171.0, 170.8, 170.5, 156.4, 156.2, 152.8, 151.0, 150.9, 148.2, 148.0, 143.9, 143.9, 140.8, 140.8, 139.1, 138.8, 127.6, 127.1, 125.7, 125.2, 125.1, 124.8, 120.2, 111.8, 111.8, 79.5, 65.5, 65.4, 50.1, 47.9, 47.6, 46.8, 46.7, 35.5, 35.2, 30.7, 28.0.

(23) Brodyagin, N.; Hnedzko, D.; MacKay, J. A.; Rozners, E. In *Peptide Nucleic Acids: Methods and Protocols*; Nielsen, P. E. (Ed.); Springer, **2020**, 157.

(24) Hansen, A. M.; Bonke, G.; Hogendorf, W. F. J.; Björkling, F.; Nielsen, J.; Kongstad, K. T.; Zabicka, D.; Tomczak, M.; Urbas, M.; Nielsen, P. E.; et al. *Eur. J. Med. Chem.* **2019**, *168*, 134.

(25) (a) Hansen, A. M.; Shaikh, A. Y.; Franzyk, H. In *Peptide Nucleic Acids: Methods and Protocols*, Nielsen, P. E. (Ed.); Springer, **2020,** 97. (b) Shaikh, A. Y.; Hansen, A. M.; Franzyk, H. In *Peptide Nucleic Acids: Methods and Protocols*, Nielsen, P. E. (Ed.); Springer, **2020**, 1.

(26) Ryan, C. A.; Rahman, M. Md; Rozners, E. *J. Am. Chem. Soc.* **2023**, *145*, 10497.