Design and Synthesis of Nucleoproline Amino Acids for the Straightforward Preparation of Chiral and Conformationally Constrained Nucleopeptides

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Dedicated to Professor *Dieter Seebach* on the occasion of his 75th birthday

A straightforward synthesis of orthogonally protected nucleoproline (Nup) amino acids and their coupling to oligomers are described. A key step is the attachment of alkynylated nucleobases to Fmoc-protected 4-azidoproline (Fmoc-Azp-OH) by a Cu-catalyzed 1,3-dipolar cycloaddition ('click reaction'). The developed protocol allows preparation of the nucleoprolines in scales of >30 g. Solid-phase peptide synthesis proved to be straightforward with these Nup amino acids. The resulting oligonucleoproline peptides adopt defined helices, are very well H_2O soluble, and show comparable cell-penetrating properties as recently reported α-nucleoalanine peptides.

Introduction. – In the last decades, the synthesis and study of synthetic nucleic acids based on a peptide skeleton has attracted considerable attention [1][2]. As a class of molecules that contain both nucleobases and amino acids, nucleopeptides are interesting for therapeutic or diagnostic applications such as the selective binding to RNA or DNA, or the design of biomaterials [1–3]. Since the invention of peptide nucleic acids (PNA) by *Nielsen et al.* [4], several different nucleopeptides have been introduced that differ mainly in the peptidic backbone, and the linker between the amino acid and the nucleobase [5]. The original PNA consists of an (2-aminoethyl)glycin backbone with acyl groups as linkers to the nucleobases (*Fig. 1*) [4]. Other nucleopeptides have α- or β-amino acids, for instance, alanine, lysine, serine, or β-alanine, in their backbone and bear the nucleobases attached to the side chains [5].

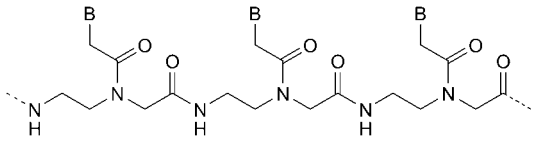


Fig. 1. *Structure of the original PNA* (B = nucleobase) [4a]

Also proline-based nucleopeptides have been investigated as constrained and preorganized PNA analogs [6]. In these nucleopeptides, the proline derivatives are

[1]) *R. A. K.* acknowledges funding by the *Roche* Postdoc Fellowship (RPF) Program.

connected by different linking units, and the nucleobases are attached at different positions of the pyrrolidine ring with an alkyl or amide linkage to the proline core. (*Fig. 2*). These derivatives are typically synthesized starting from naturally occurring *trans*-hydroxy-L-proline. Several of the resulting chiral, constrained, and structurally preorganized nucleopeptides have improved binding affinities towards DNA or RNA compared to the original PNA, and discriminate between parallel and antiparallel binding orientation [6].

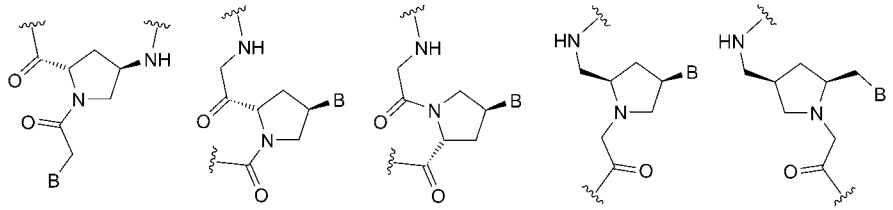


Fig. 2. *Examples of proline-containing PNA analogs* (B = nucleobase) [6]

We have recently introduced azidoproline (Azp)-containing oligoprolines (*Fig. 3*) as conformationally well-defined molecular scaffolds that can be easily functionalized with alkynes using Cu-catalyzed 1,3-dipolar cycloaddition [7] or by amidation with carboxylic acid derivatives after reduction of the azides to amines [8]. In an aqueous environment, these oligomers adopt the highly symmetric polyproline II (PPII) helix, in which every third residue is stacked on top of each other at a distance of *ca.* 1 nm (*Fig. 3*) [8][9]. This conformationally well-defined helix is already adopted at chain lengths as short as six proline residues. All of these features render functionalizable oligoprolines intriguing for manifold different applications such as the controlled formation of silver nanoparticles [10], studies of electron transfer [11], or the development of functionalized synthetic collagen [12].

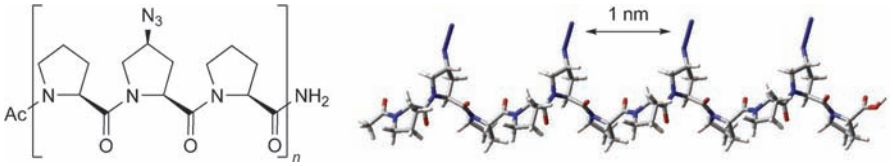
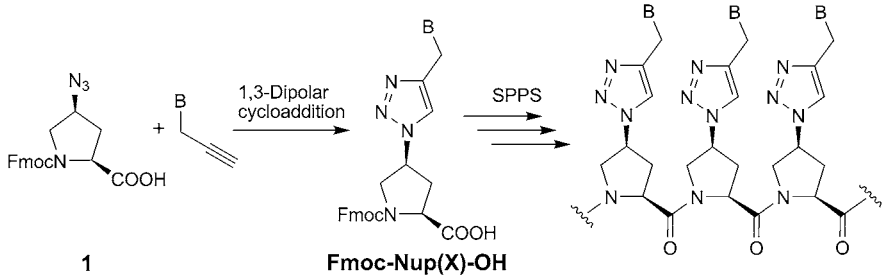


Fig. 3. *Structure of oligoprolines functionalized in every third position with N_3 groups*

The synthesis of such functionalizable oligoprolines is straightforward using Fmoc-Pro-OH and Fmoc-Azp-OH (**1**) as the functionalizable building block, that can be easily prepared in large scale and has excellent coupling efficiencies in peptide synthesis [8].

We envisaged chiral and conformationally constrained oligonucleoprolines, with yet a different geometry and thereby coordination properties compared to previously developed nucleopeptides, as useful for the selective binding to RNA or DNA, and the construction of tailor-made supramolecular assemblies. Towards the synthesis of such nucleoproline (Nup) peptides by standard solid-phase peptide synthesis (SPPS) following the Fmoc/^{t}Bu protocol, appropriately protected nucleobase proline amino acids, Fmoc-Nup(X)-OH, are required (*Scheme 1*). The key step for the synthesis of

Scheme 1. *Synthesis Strategy of Nucleobase-Modified Proline Derivatives, Fmoc-Nup(X)-OH, and Solid-Phase Peptide Synthesis* (SPPS) *of Oligonucleoprolines*



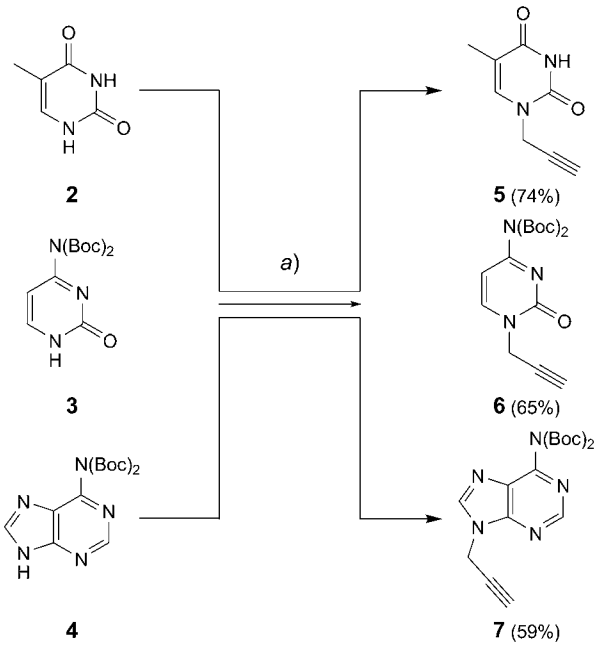
these Fmoc-Nup-OH amino acids is the attachment of alkyne-functionalized nucleobases to azidoproline **1** *via* a Cu-catalyzed 1,3-dipolar cycloaddition reaction ('click reaction') [7].

Results and Discussion. – To avoid undesired side-reactions in the synthesis of the α-Fmoc-protected Nup monomers, Fmoc-Nup(X)-OH, the exocyclic amines of cytosine, adenine, and guanine had to be masked with protecting groups that are orthogonal to the Fmoc protecting group and are compatible with SPPS. No protection was necessary for thymine, since it lacks an exocyclic amine group. A general problem for the synthesis of nucleopeptides is the often low solubility of the nucleobase-containing building blocks. *Porcheddu et al.* showed that protection of the exocyclic amines of the nucleobases with two Boc groups, increases the lipophilicity of the nucleobases and thereby their solubility in organic solvents [13]. We decided to use the same protection-group strategy and hence synthesized the bis-Boc-protected nucleobases Boc-cytosine **3** and Boc-adenine **4** in multi-gram scale, according to the known procedure (*Scheme 2*) [13b]. This involved tris-Boc protection of cytosine and adenine, followed by a selective mono-Boc deprotection to yield **3** and **4**, respectively.

The alkylation of thymine **2**, and the protected cytosine and adenine derivatives, **3** and **4**, was achieved using K_2CO_3 and propargyl bromide in DMF to obtain the corresponding functionalized nucleobases **5**, **6**, and **7**, respectively, ready for the attachment to azidoproline **1** (*Scheme 2*).

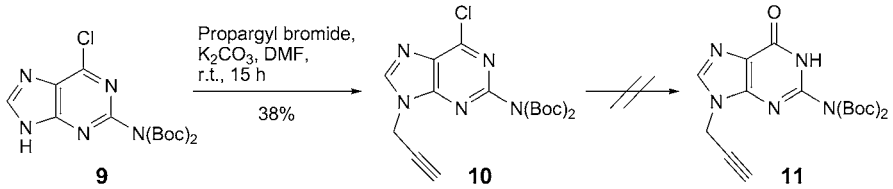
The development of an efficient synthesis route to a suitably protected, alkynylated guanine derivative presented the most challenging part of the Nup synthesis. To overcome the low reactivity and solubility of guanine, we initially used the more reactive 6-chloroguanine (**8**) [14] and increased its solubility with the bis-Boc-protection strategy. Haloguanine derivative **9** was synthesized according to the same procedure described for the synthesis of the cytosine and adenine derivatives **3** and **4** [13b], followed by its alkylation with propargyl bromide to yield the alkynylated 6-haloguanine derivative **10** (*Scheme 3*). Several known methods for the final conversion of **10** to the protected guanine **11** were then evaluated, *e.g.*, reaction of the 6-halo group with O-nucleophiles such as 3-hydroxypropanenitrile in combination with NaH, 2-cyanoethanol in combination with NaH, Me_3N in combination with H_2O, or Me_3N in the presence of 3-hydroxypropanenitrile and DBU (=1,8-diazabicyclo[5.4.0]undec-7-

Scheme 2. *Synthesis of Alkyne-Modified Thymine, Cytosine, and Adenine Derivatives* **5**, **6**, *and* **7**, *Respectively*



a) Propargyl bromide, K_2CO_3, DMF, r.t., 15 h.

Scheme 3. *Tentative Synthetis of Bis-Boc-Protected Guanine Derivative* **11**

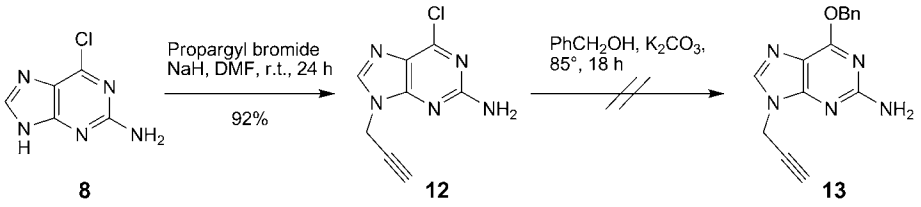


ene) [13][15]. Unfortunately, all of these trials resulted either in the decomposition of **10** or in complete recovery of the starting material.

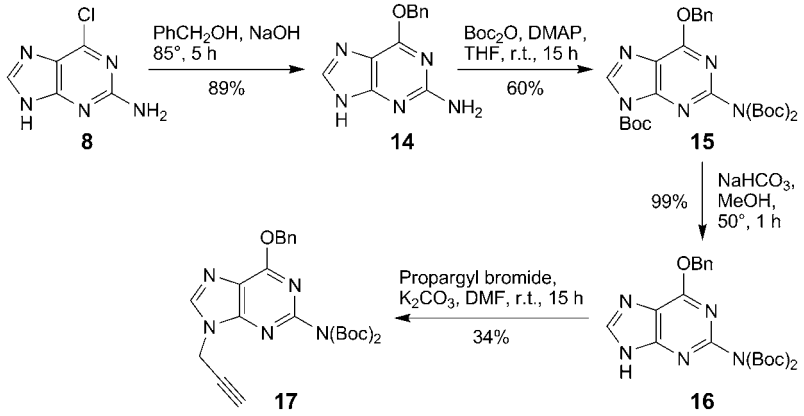
We, therefore, focused our attention on the use of *O*(*6*)-benzyl (Bn)-protected guanine derivatives as starting material, since the Bn protecting group can be cleaved under the acidic conditions used for cleaving the Boc groups [16]. In a first attempt, we alkylated 6-chloroguanine (**8**) with propargyl bromide to yield the haloguanine derivative **12**, which, however, did not react with BnOH to the desired guanine derivative **13** (*Scheme 4*) [17].

The successful route to the desired alkynylated guanine building block **17** started by converting 6-chloroguanine (**8**) with BnOH to 6-(benzyloxy)guanine (**14**; *Scheme 5*) [18]. Guanine derivative **14** was then reacted to the tris-Boc-protected derivative **15**

Scheme 4. *Tentative Synthetis of Benzyl* (Bn)*-Protected Guanine* **13**



Scheme 5. *Synthesis of Alkynylated Guanine Derivative* **17** (DMAP = 4-(dimethylamino)pyridine)

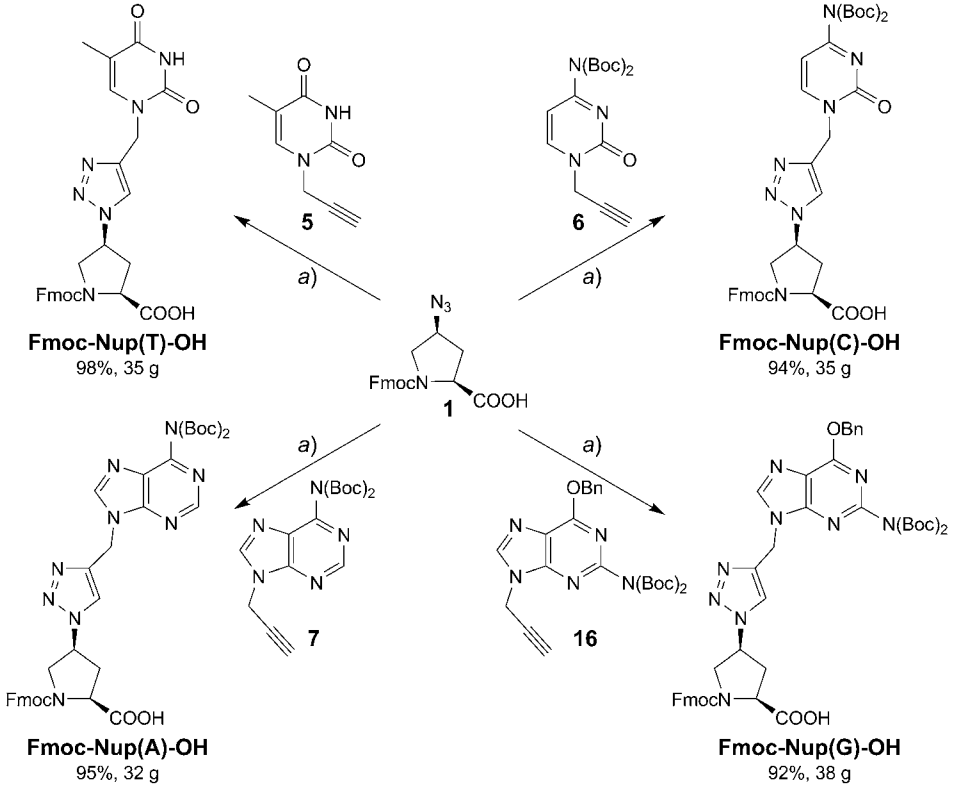


with Boc_2O and catalytic amounts of DMAP, followed by selective Boc deprotection at N(9) with aqueous $NaHCO_3$ in MeOH to yield bis-Boc-protected guanine derivative **16**. The final alkylation with propargyl bromide resulted in the desired guanine derivative **17**, ready for the attachment to azidoproline **1**. This route allowed preparation of the bis-Boc-protected and alkynylated building block **17** in a scale of 22 g.

For the conjugation of Fmoc-Azp-OH (**1**) with the alkyne-functionalized nucleobases **5**, **6**, **7**, and **17**, several Cu salts, *e.g.*, $CuSO_4 \cdot 5\,H_2O$, $Cu(OAc)_2$, CuI, and $[Cu(MeCN)_4]PF_6$, in the presence or absence of sodium ascorbate as reducing agent and different conditions (solvents, temperatures, *etc.*) for the 1,3-dipolar cycloaddition reaction were tested (*Scheme 6*) [7]. Nearly quantitative conversion in multi-gram scale was obtained with the very well DMF soluble Cu^I complex $[Cu(MeCN)_4]PF_6$ at 45°, whereas the other tested copper salts did either not allow for reaction or provided the products in poor yields. Using these optimized conditions allowed for the synthesis of all four nucleoproline derivatives in yields of $>92\%$ and quantities of >30 g (*Scheme 6*).

With all four building blocks in hand, we next studied their coupling efficiencies in SPPS following the Fmoc/^{t}Bu protocol. The monomers Fmoc-Nup(A)-OH, Fmoc-Nup(T)-OH, and Fmoc-Nup(C)-OH showed comparable coupling yields as standard Fmoc-protected α-amino acids. Six consecutive coupling steps with the Nup derivatives

Scheme 6. *Synthesis of the α-Fmoc-Protected Nucleoproline Derivatives Fmoc-Nup(X)-OH*



a) $[Cu(MeCN)_4]PF_6$, DMF, 45°, 2 h.

using COMU (=1-{[(1-cyano-2-ethoxy-2-oxoethylidene)aminooxy](dimethylamino)-morpholino}uronium hexafluorophosphate) [19] as coupling reagent allowed for preparing the hexamers Ac-[Nup(A)]$_6$-NH_2, Ac-[Nup(T)]$_6$-NH_2, and Ac-[Nup(C)]$_6$-NH_2 in purities of $>94\%$, as judged by HPLC analysis. This corresponds to an average coupling yield of 99% for each coupling step. The purity of the hexamer, Ac-[Nup(G)]$_6$-NH_2, prepared under identical conditions, was only *ca.* 65% corresponding to an average coupling yield of 93% for Fmoc-Nup(G)-OH. The lower coupling efficiencies of the guanine building block in this all-Nup(G) nucleopeptide might be due to the steric hindrance of the bulky Nup(G) monomers. Reassuringly, when Nup(G) was incorporated into mixed nucleopeptides such as Ac-Nup(G)-Nup(C)-Nup(A)-Nup(T)-Nup(C)-Nup(G)-Nup(A)-NH_2 (**18**), also with Fmoc-Nup(G)-OH, excellent average coupling efficiencies of *ca.* 99% for each coupling step were achieved to yield this peptide in a purity of 94% (*Fig. 4*).

Similar coupling efficiencies were obtained for mixed proline and Nup peptides, for example, H-Lys(CF)-[Pro-Nup(T)-Pro]$_4$-Lys-NH_2 (**19**). Conformational analysis of this peptide by circular dichroism (CD) spectroscopy showed that this nucleobase-

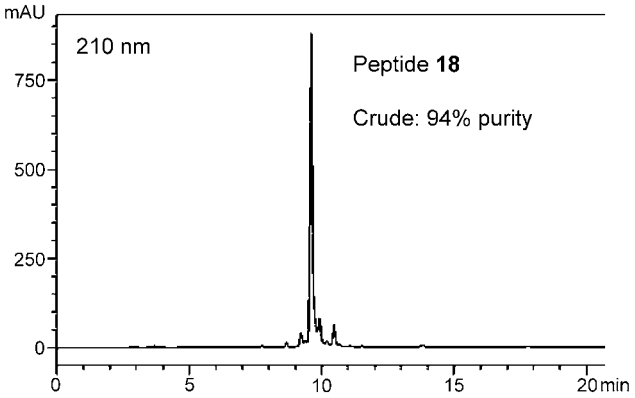


Fig. 4. *HPLC Chromatogram of crude Ac-Nup(G)-Nup(C)-Nup(A)-Nup(T)-Nup(C)-Nup(G)-Nup-(A)-NH_2* (**18**)

functionalized peptide adopts a polyproline type-II conformation in aqueous environments, as indicated by the minima and maxima at wavelengths of 205 and 227 nm, respectively (*Fig. 5*) [9][10]. This demonstrates that the triazole nucleobase units do not influence the conformational preferences of oligoprolines significantly.

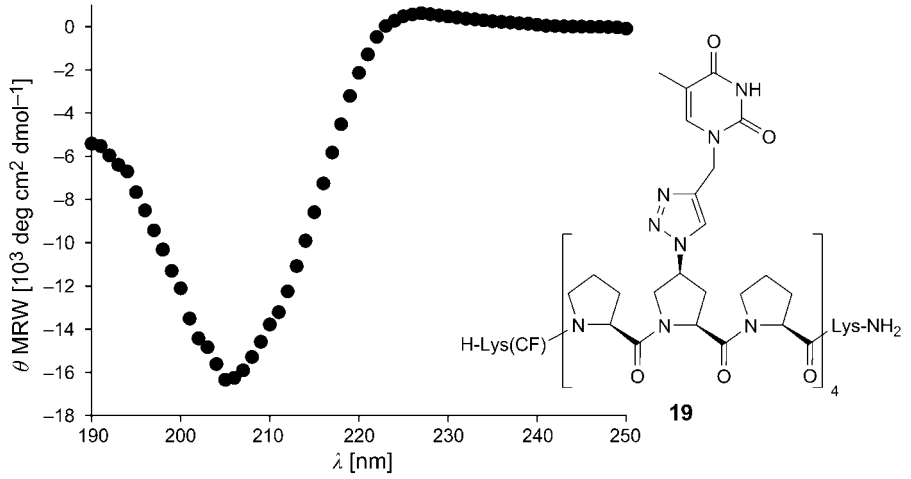


Fig. 5. *CD Spectrum of mixed proline and nucleoproline peptide H-Lys(CF)-[Pro-Nup(T)-Pro]$_4$-Lys-NH_2* (**19**; CF = 5(6)-carboxyfluorescein)

We have also investigated the solubility of such artificial Nup analogs. As a representative, the hexamer $[Nup(T)]_6$ was chosen which showed a remarkably high solubility of > 3000 ug/ml.

For biological applications of nucleopeptides for, *e.g.*, DNA or RNA targeting, a big challenge is their transport to the target molecules through the cell membrane into the cells [1b][20]. Thus, often cell-penetrating peptides such as polyarginines [21] or their β-amino acid analogs [22] are used as carriers to facilitate the cellular uptake.

Nucleoalanines Ala(T) conjugated to polyalanines have been reported by *Formaggio*, *Bianco* and co-workers to penetrate the cell membrane without the help of a molecular transporter [5g]. We compared the cell-penetrating properties of the reported oligonucleoalanine peptide **20**, with those of oligonucleoproline peptide **21**, in which the nucleoalanine Ala(T) moieties of peptide **20** were replaced with nucleoprolines, Nup(T), and additionally with those of oligonucleoproline peptide **19**, in which all Ala(T) moieties and alanine units of **20** are exchanged with Nup(T) and proline, respectively. The peptides were labeled with the fluorophore 5(6)-carboxyfluorescein (CF). Incubation of HeLa cells for 1 h with the different peptides and additionally with CF as a control at concentrations of 50 μM, followed by fluorescence activated cell sorting (FACS) studies, allowed determination of the relative cellular uptake of the peptides into the cells (*Fig. 6*).

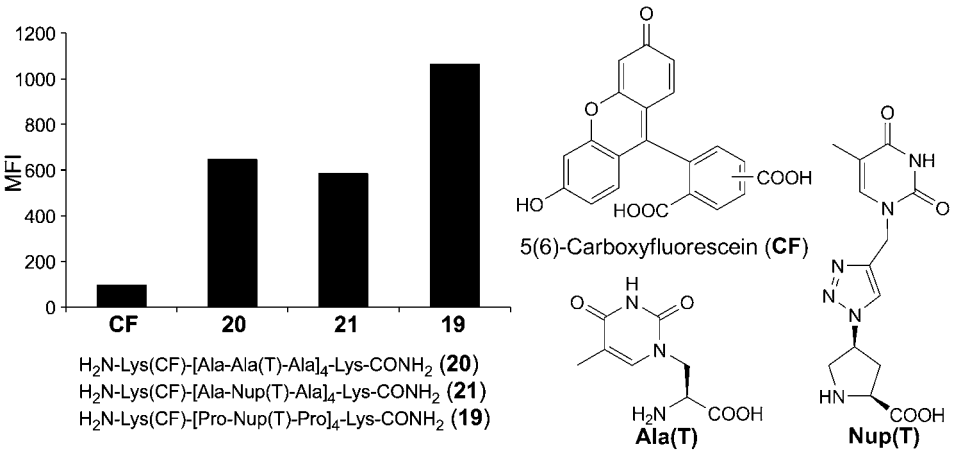


Fig. 6. *Cellular uptake of peptides* **19 – 21** (MFI = mean fluorescence intensity)

The results show that the uptake efficiencies of oligonucleoprolines are at least comparable to those of the reference compound **20** (*Fig. 6*). Nonetheless, these results have to be taken with caution, since cellular uptake is only observed at very high and non-physiologically relevant concentrations of 50 μM for all of the peptides.

Conclusions. – We have developed a straightforward synthesis protocol for the preparation of orthogonally protected nucleoproline monomers. Key step is the attachment of the alkyne-functionalized nucleobases to Fmoc-Azp-OH by a 1,3-dipolar cycloaddition reaction, that proceeds in yields of > 92% on a 30-g scale. The resulting nucleoproline amino acids have excellent coupling efficiencies in SPPS rendering them attractive for the preparation of chiral, structurally constrained nucleobase-modified peptides. The CD spectrum of a mixed proline and nucleoproline peptide showed that the triazole nucleobase units do not influence the polyproline type-II conformation significantly. These structurally constrained nucleoproline peptides might, therefore, be useful for applications in targeting RNA or DNA, or for the construction of tailor-made supramolecular assemblies. In addition, nucleoprolines have good solubility and

comparable cellular-uptake efficiencies in HeLa cells as previously reported α-nucleoalanines.

Experimental Part

General. Materials and reagents were of the highest commercially available grade and used without further purification. Compounds **3**, **4**, and **14** were synthesized according to the procedures reported by *Porcheddu et al.* [13b]. Reactions were monitored by TLC using *Merck* silica gel (SiO_2) *60 F254* plates; visualization by UV and ninhydrin. Anal. HPLC: *Reprosil Gold 120 C18 RP* 5 μm (150 mm × 4 mm) column from *Dr. Maisch GmbH*. Prep. HPLC: *Reprosil Gold 120 C18 RP* 5 μm (150 mm × 16 mm) column from *Dr. Maisch GmbH*. Flash column chromatography (CC): *Merck* SiO_2 *60* or *Fluka* SiO_2 *60*, particle size 40–63 μm. CD Spectra: *Chirascan* spectrometer (*Applied Biophysics Ltd*, Leatherhead, UK). ^{1}H- and ^{13}C-NMR spectra: *Bruker DPX 400* spectrometer; chemical shifts in ppm, with the solvent signal as a reference. ESI-MS: *Bruker solariX 94* instrument.

tert-*Butyl 6-(Benzyloxy)-2-[[bis(*tert-*butoxy)carbonyl]amino}-9*H-*purine-9-carboxylate* (**15**). To a suspension of *6-(benzyloxy)-9*H-*purin-2-amine* (**14**; 50.0 g, 207 mmol) and DMAP (2.30 g, 20.7 mmol, 0.1 equiv.) in THF was added Boc_2O (130 g, 745 mmol, 3.6 equiv.). The mixture was stirred overnight at r.t. The excess amount of THF was removed at reduced pressure, and the crude product was dissolved in CH_2Cl_2 (1500 ml), washed with HCl aq. (1N, 300 ml) and brine (2 × 300 ml), dried (Na_2SO_4), and all volatiles were removed at reduced pressure. The remaining residue was purified by CC (SiO_2; CH_2Cl_2 → CH_2Cl_2/MeOH 99:1) to give **15** (67.1 g, 124 mmol, 60%). Colorless solid. R_f (CH_2Cl_2/MeOH 98:2) 0.53. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.76 (*s*, 1 H); 7.57–7.52 (*m*, 2 H); 7.47–7.37 (*m*, 3 H); 5.64 (*s*, 2 H); 1.66 (*s*, 9 H); 1.46 (*s*, 18 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 153.2; 152.7; 151.2; 150.8; 148.0; 145.9; 131.2; 129.4; 129.4; 87.6; 84.5; 84.1; 83.9; 31.9; 28.3; 28.3. HR-ESI-MS: 564.2422 ($[M+Na]^+$, $C_{27}H_{35}N_5NaO_7^+$; calc. 564.2434).

*Di(*tert-*butyl) [6-(Benzyloxy)-9*H-*purin-2-yl]imidodicarbonate* (**16**). To a soln. of **15** (50 g, 92.4 mmol) in MeOH (1000 ml) was added sat. aq. $NaHCO_3$ (450 ml). The turbid soln. was stirred at 50° for 1 h. After removal of MeOH at reduced pressure, H_2O (500 ml) was added, and the aq. suspension was extracted with CH_2Cl_2 (3 × 500 ml). The combined org. layers were dried (Na_2SO_4), filtered, and all volatiles were removed at reduced pressure to give pure **16** (40.4 g, 91.3 mmol, 99%). Colorless solid. R_f (CH_2Cl_2/MeOH 98:2) 0.10. ^{1}H-NMR (400 MHz, (D_6)DMSO): 14.11 (*s*, 1 H); 8.49 (*s*, 1 H); 7.57–7.52 (*m*, 2 H); 7.46–7.34 (*m*, 3 H); 5.63 (*s*, 2 H); 1.44 (*s*, 18 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 153.1; 152.2; 151.6; 151.4; 145;2; 129.4; 129.3; 84.1; 83.5; 55.8; 31.9; 28.3. HR-ESI-MS: 464.1901 ($[M+H]^+$, $C_{22}H_{27}N_5NaO_5^+$; calc. 464.1910).

*Di(*tert-*Butyl) [6-(Benzyloxy)-9-(prop-2-yn-1-yl)-9*H-*purin-2-yl]imidodicarbonate* (**17**). To a suspension of **16** (59.6 g, 135 mmol) and anh. K_2CO_3 (18.7 g, 135 mmol, 1.0 equiv.) in DMF (400 ml), was added propargyl bromide (80 wt-% in toluene, 54.5 ml, 405 mmol, 3.0 equiv.). The mixture was stirred at r.t. for 15 h. After removal of the solvent at reduced pressure, the residue was suspended in H_2O (500 ml). The aq. soln. was extracted with CH_2Cl_2 (3 × 500 ml), the combined org. layers were dried (Na_2SO_4), and all volatiles were removed at reduced pressure. The remaining residue was purified by CC (SiO_2; AcOEt/hexane 2:3 → 3:2) to give **17** (22.1 g, 46.1 mmol, 34%). Colorless solid. R_f (AcOEt/hexane 3:2) 0.35. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.55 (*s*, 1 H); 7.52–7.57 (*m*, 2 H); 7.37–7.46 (*m*, 3 H); 5.66 (*s*, 2 H); 5.17 (*d*, *J* = 2.6, 2 H); 3.55 (*t*, *J* = 2.5, 1 H); 1.42 (*s*, 18 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 161.0; 153.4; 152.0; 151.3; 145.3; 136.8; 129.4; 129.2; 129.2; 119.9; 83.7; 78.7; 77.1; 69.1; 33.8; 28.3. HR-ESI-MS: 502.2067 ($[M+Na]^+$, $C_{25}H_{29}N_5NaO_5^+$; calc. 502.2066).

*5-Methyl-1-(prop-2-yn-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione* (**5**). To a suspension of thymine (**2**; 15.0 g, 119 mmol) and anh. K_2CO_3 (16.5 g, 119 mmol, 1.0 equiv.) in DMF (400 ml) was added propargyl bromide (80 wt-% in toluene, 48.0 ml, 357 mmol, 3.0 equiv.). The mixture was stirred at r.t. for 15 h. After removal of the solvent at reduced pressure, the residue was suspended in H_2O (500 ml). The aq. soln. was extracted with CH_2Cl_2 (3 × 500 ml), and the combined org. layers were dried (Na_2SO_4), and all volatiles were removed at reduced pressure. The remaining residue was purified by CC (SiO_2; AcOEt/hexane

2:3 → 3:2) to give **5** (14.4 g, 88.1 mmol, 74%). Colorless solid. R_f (AcOEt/hexane 3:2) 0.35. The spectroscopic data are in accordance to those reported in [23].

*Di(*tert*-butyl) [2-(Prop-2-ynoyl)pyrimidin-4-yl]imidodicarbonate* (**6**). To a suspension of **3** (30.0 g, 96.4 mmol) and anh. K_2CO_3 (13.3 g, 96.4 mmol, 1.0 equiv.) in DMF (300 ml) was added propargyl bromide (80 wt-% in toluene, 38.8 ml, 289 mmol, 3.0 equiv.). The mixture was stirred at r.t. for 15 h. After removal of the solvent at reduced pressure, the residue was suspended in H_2O (500 ml). The aq. soln. was extracted with CH_2Cl_2 (3 × 500 ml), the combined org. layers were dried (Na_2SO_4), and all volatiles were removed at reduced pressure. The remaining residue was purified by CC (SiO_2; AcOEt/hexane 2:3 → 1:1) to give **6** (21.9 g, 62.7 mmol, 65%). Colorless solid. R_f (AcOEt) 0.54. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.29 (*d*, *J* = 7.3, 1 H); 6.89 (*d*, *J* = 7.3, 1 H); 4.70 (*d*, *J* = 2.5, 2 H); 3.52 (*t*, *J* = 2.5, 1 H); 1.53 (*s*, 18 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 162.8; 154.3; 150.5; 150.1; 96.9; 85.5; 78.9; 77.6; 39.7; 28.1. HR-ESI-MS: 372.1543 ($[M+Na]^+$, $C_{17}H_{23}N_3NaO_5^+$; calc. 372.1535).

*Di(*tert*-butyl) [9-(Prop-2-yn-1-yl)-9*H*-purin-6-yl]imidodicarbonate* (**7**). To a suspension of **4** (30.0 g, 89,5 mmol) and anh. K_2CO_3 (12.3 g, 89.5 mmol, 1.0 equiv.) in DMF (300 ml) was added propargyl bromide (80 wt-% in toluene, 36.1 ml, 269 mmol, 3.0 equiv.). The mixture was stirred at r.t. for 15 h. After removal of the solvent at reduced pressure, the residue was suspended in H_2O (500 ml). The aq. soln. was extracted with CH_2Cl_2 (3 × 500 ml), the combined org. layers were dried (Na_2SO_4), and all volatiles were removed at reduced pressure. The remaining residue was purified by CC (SiO_2; AcOEt/hexane 2:3 → 1:1) to give **7** (19.7 g, 52.8 mmol, 59%). Colorless solid. R_f (AcOEt) 0.59. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.93 (*s*, 1 H); 8.74 (*s*, 1 H); 5.26 (*d*, *J* = 2.5, 2 H); 3.58 (*t*, *J* = 2.5, 1 H); 1.42 (*s*, 18 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 153.5; 152.6; 150.9; 150.1; 147.1; 128.5; 84.3; 78.5; 77.4; 34.0; 28.2. HR-ESI-MS: 396.1634 ($[M+Na]^+$, $C_{18}H_{23}N_5NaO_4^+$; calc. 396.1648).

*(4*S*)-1-{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-proline* (Fmoc-Nup(T)-OH). A soln. of **5** (10.9 g, 66.7 mmol) and *Fmoc-Azp-OH* (**1**; 25.2 g, 66.7 mmol, 1.0 equiv.) in DMF (500 ml) was degassed in an ultrasonic bath for 20 min, followed by the addition of $[Cu(MeCN)_4]PF_6$ (1.24 g, 3.34 mmol, 5 mol-%). The soln. was stirred at 45° for 2 h. After removal of the solvent at reduced pressure, the residue was suspended in a sat. soln. of EDTA (= ethylenediaminetetraacetic acid) in H_2O (0.5M, 500 ml).The aq. soln. was extracted with CH_2Cl_2 (3 × 1000 ml), and the combined org. layers were washed with aq. sat. NaCl (1000 ml), dried (Na_2SO_4), and all volatiles were removed at reduced pressure. The remaining residue was purified by CC (SiO_2; AcOEt/hexane 2:3 → 3:2) to give Fmoc-Nup(T)-OH (35.5 g, 65.5 mmol, 98%). Colorless solid. R_f (AcOEt/hexane 3:2) 0.35. The NMR spectra indicated two conformers (*cis* and *trans* conformation around the carbamate bound) in a ratio of 1:2.1. ^{1}H-NMR (400 MHz, (D_6)DMSO): major conformer: 11.35 (*s*, NH); 8.28 (*s*, H–C(6) thymine); 7.95 – 7.89 (*m*, 2 H of Fmoc); 7.75 – 7.69 (*m*, 2 H of Fmoc); 7.63 (*s*, 1 H of triazole); 7.48 – 7.41 (*m*, 2 H of Fmoc); 7.40 – 7.32 (*m*, 2 H of Fmoc); 5.33 – 5.22 (*m*, H–C_γ(Pro)); 4.94 (*s*, CH_2 of thymine); 4.12 – 4.38 (*m*, H–C_α(Pro), CH_2 of Fmoc, CH of Fmoc, H–C_β(Pro)); 3.73 – 3.84 (*m*, H–C_β(Pro)); 2.90 – 3.01 (*m*, H–C_δ(Pro)); 2.41 – 2.53 (*m*, H–C_δ(Pro)); 1.79 (*s*, Me of thymine); minor conformer: 11.35 (*s*, NH); 8.28 (*s*, H–C(6) thymine); 7.95 – 7.89 (*m*, 2 H of Fmoc); 7.75 – 7.69 (*m*, 2 H of Fmoc); 7.63 (*s*, 1 H of triazole); 7.48 – 7.41 (*m*, 2 H of Fmoc); 7.40 – 7.37 (*m*, 2 H of Fmoc); 5.33 – 5.28 (*m*, H–C_γ(Pro)); 4.94 (*s*, CH_2 of thymine); 4.54 (*dd*, *J* = 7.4, H–C_α(Pro)); 4.12 – 4.38 (*m*, CH_2 of Fmoc, CH of Fmoc, H–C_β(Pro)); 3.73 – 3.84 (*m*, H–C_β(Pro)); 3.01 – 3.11 (*m*, H–C_δ(Pro)); 2.52 – 2.63 (*m*, H–C_δ(Pro)); 1.79 (*s*, Me of thymine). ^{13}C-NMR (101 MHz, (D_6)DMSO): major conformer: 165.3; 151.8; 144.8; 143.7; 142.1; 141.7; 128.8; 128.2; 126.4; 126.2; 124.1; 121.2; 109.9; 68.3; 68.0; 59.1; 58.8; 57.6; 53.8; 52.4; 51.8; 47.7; 47.6; 43.1; 35.6; 35.6; minor conformer: 165.3; 151.8; 144.8; 143.7; 142.1; 141.7; 128.8; 128.2; 126.4; 126.2; 124.1; 121.2; 109.9; 68.3; 68.0; 59.1; 58.4; 57.6; 53.8; 52.4; 51.8; 47.7; 47.6; 43.1; 36.8; 36.7. HR-ESI-MS: 543.1990 ($[M+H]^+$, $C_{28}H_{27}N_6O_6^+$; calc. 543.1992).

*(4*S*)-4-{4-[(4-{[Bis(*tert*-butoxy)carbonyl]amino}-2-oxopyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-1-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-*L*-proline* (Fmoc-Nup(C)-OH). A soln. of **6** (17.4 g, 49.9 mmol) and **1** (18.9 g, 49.9 mmol, 1.0 equiv.) in DMF (500 ml) was degassed in an ultrasonic bath for 20 min, followed by the addition of $[Cu(MeCN)_4]PF_6$ (0.928 g, 2.50 mmol, 5 mol-%). The soln. was stirred at 45° for 2 h. After removal of the solvent, the residue was suspended in a sat. soln. of EDTA in H_2O (0.5M, 500 ml). The aq. soln. was extracted with CH_2Cl_2 (3 × 1000 ml), the combined org. layers were washed with aq. sat NaCl (1000 ml), dried (Na_2SO_4), and all volatiles were removed at reduced

pressure. The remaining residue was purified by CC (SiO_2; AcOEt/hexane 2:3 → 3:2) to give Fmoc-Nup(C)-OH (34,8 g, 46.9 mmol, 94%). Colorless solid. R_f (AcOEt/hexane 3:2) 0.35. The NMR spectra indicated two conformers (*cis* and *trans* conformation around the carbamate bound) in a ratio of 1:1.8. ^{1}H-NMR (400 MHz, (D_6)DMSO): major conformer: 8.33–8.29 (*m*, 1 H of cytosine); 7.98 (*s*, 1 H of triazole); 7.91–7.89 (*m*, 2 H of Fmoc); 7.73–7.68 (*m*, 2 H of Fmoc); 7.47–7.42 (*m*, 2 H of Fmoc); 7.40–7.33 (*m*, 2 H of Fmoc); 6.90–6.86 (*m*, 1 H of cytosine); 5.35–5.23 (*m*, H–C_γ(Pro)); 5.14 (*s*, CH_2 of triazole); 4.13–4.40 (*m*, H–C_α(Pro), CH_2 of Fmoc, CH of Fmoc, H–C_β(Pro)); 3.76–3.85 (*m*, H–C_β(Pro)); 2.94–3.01 (*m*, H–C_δ(Pro)); 2.45–2.56 (*m*, H–C_δ(Pro)); 1.51 (*s*, 2 Me_3C); minor conformer: 8.33–8.29 (*m*, 1 H of cytosine); 7.98 (*s*, 1 H of triazole); 7.91–7.89 (*m*, 2 H of Fmoc); 7.73–7.68 (*m*, 2 H of Fmoc); 7.47–7.42 (*m*, 2 H of Fmoc); 7.40–7.33 (*m*, 2 H of Fmoc); 6.90–6.86 (*m*, 1 H of cytosine); 5.33–5.23 (*m*, H–C_γ(Pro)); 5.14 (*s*, CH_2 of triazole); 4.59–4.51 (*m*, H–C_α(Pro)); 4.13–4.40 (*m*, CH_2 of Fmoc, CH of Fmoc, H–C_β(Pro)); 3.76–3.85 (*m*, H–C_β(Pro)); 3.01–3.11 (*m*, H–C_δ(Pro)); 2.57–2.66 (*m*, H–C_δ(Pro)); 1.51 (*s*, 2 Me_3C). ^{13}C-NMR (101 MHz, (D_6)DMSO): major conformer: 163.3; 162.8; 154.9; 154.8; 151.6; 150.3; 144.7; 142.9; 141.7; 128.7; 128.2; 126.3; 126.2; 124.5; 121.2; 96.7; 85.6; 68.3; 68.0; 58.8; 58.4; 52.4; 51.8; 47.7; 47.6; 45.5; 35.49; 35.2; 38.3; 28.3; minor conformer: 163.3; 162.8; 154.9; 154.8; 151.6; 150.3; 144.7; 142.9; 141.7; 128.8; 128.2; 126.3; 126.6; 124.5; 121.1; 96.7; 85.6; 68.3; 68.0; 58.8; 57.6; 52.4; 51.8; 47.7; 47.6; 45.5; 36.6; 36.4; 28.3. HR-ESI-MS: 750.2858 ([M + Na]$^+$, $C_{37}H_{41}N_7NaO_9^+$; calc. 750.2863).

*(4*S*)-4-[4-({6-[Bis(*tert*-butoxycarbonyl)amino]-9*H*-purin-9-yl}methyl)-1*H*-1,2,3-triazol-1-yl]-1-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-*L*-proline* (Fmoc-Nup(A)-OH). A soln. of **7** (16.6 g, 44.5 mmol) and **1** (16.8 g, 44.5 mmol, 1.0 equiv.) in DMF (500 ml) was degassed in an ultrasonic bath for 20 min, followed by the addition of [Cu(MeCN)$_4$]PF$_6$ (0.828 g, 2.23 mmol, 5 mol-%). The soln. was stirred at 45° for 2 h. After removal of the solvent, the residue was suspended in a sat. soln. of EDTA in H_2O (0.5M, 500 ml). The aq. soln. was extracted with CH_2Cl_2 (3 × 1000 ml), the combined org. layers were washed with aq. sat. NaCl (1000 ml), dried (Na_2SO_4), and all volatiles were removed at reduced pressure. The remaining residue was purified by CC (SiO_2; AcOEt/hexane 2:3 → 3:2) to give Fmoc-Nup(A)-OH (31.7 g, 42.3 mmol, 95%). Colorless solid. R_f (AcOEt/hexane 3:2) 0.35. The NMR spectra indicated two conformers (*cis* and *trans* conformation around the carbamate bound) in a ratio of 1:1.9. ^{1}H-NMR (400 MHz, (D_6)DMSO): major conformer: 8.74 (*s*, 1 H of adenine); 8.44 (*s*, 1 H of adenine); 7.99 (*s*, 1 H of triazole); 7.94–7.89 (*m*, 2 H of Fmoc); 7.75–7.67 (*m*, 2 H of Fmoc); 7.47–7.41 (*m*, 2 H of Fmoc); 7.39–7.32 (*m*, 2 H of Fmoc); 5.65 (*s*, CH_2 of triazole); 5.34–5.23 (*m*, H–C_γ(Pro)); 4.12–4.38 (*m*, H–C_α(Pro), CH_2 of Fmoc, CH of Fmoc, H–C_β(Pro)); 3.73–3.84 (*m*, H–C_β(Pro)); 2.89–2.99 (*m*, H–C_δ(Pro)); 2.41–2.52 (*m*, H–C_δ(Pro)); 1.42 (*s*, 2 Me_3C); minor conformer: 8.74 (*s*, 1 H of adenine); 8.44 (*s*, 1 H of adenine); 7.99 (*s*, 1 H of triazole); 7.94–7.89 (*m*, 2 H of Fmoc); 7.75–7.67 (*m*, 2 H of Fmoc); 7.47–7.41 (*m*, 2 H of Fmoc); 7.39–7.32 (*m*, 2 H of Fmoc); 5.65 (*s*, CH_2 of triazole); 5.34–5.23 (*m*, H–C_γ(Pro)); 4.56–4.50 (*m*, H–C_α(Pro)); 4.12–4.38 (*m*, CH_2 of Fmoc, CH of Fmoc, H–C_β(Pro)); 3.73–3.84 (*m*, H–C_β(Pro)); 3.01–2.99 (*m*, H–C_δ(Pro)); 2.56–2.63 (*m*, H–C_δ(Pro)); 1.42 (*s*, 2 Me_3C). ^{13}C-NMR (101 MHz, (D_6)DMSO): major conformer: 163.4; 153.9; 152.6; 151.2; 150.1; 147.8; 144.8; 142.8; 141.7; 128.8; 128.6; 128.2; 126.4; 126.2; 124.3; 121.2; 68.3; 67.9; 59.3; 59.0; 58.5; 57.8; 52.5; 51.9; 47.7; 47.6; 35.7; 35.2; 35.0; 28.3; minor conformer: 163.4; 153.9; 152.6; 151.2; 150.1; 147.8; 144.8; 142.8; 141.7; 128.8; 128.6; 128.2; 126.4; 126.2; 124.3; 121.2; 68.3; 67.9; 59.3; 59.0; 58.5; 57.8; 52.5; 51.9; 47.7; 47.6; 36.1; 36.0; 28.3. HR-ESI-MS: 774.2980 ([M + Na]$^+$, $C_{38}H_{41}N_9NaO_8^+$; calc. 774.2976).

*(4*S*)-4-[4-({6-(Benzyloxy)-2-[bis(*tert*-butoxycarbonyl)amino]-9*H*-purin-9-yl}methyl)-1*H*-1,2,3-triazol-1-yl]-1-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-*L*-proline* (Fmoc-Nup(G)-OH). A soln. of **17** (23,4 g, 48.8 mmol) and **1** (18.5 g, 48.8 mmol, 1.0 equiv.) in DMF (500 ml) was degassed in an ultrasonic bath for 20 min, followed by the addition of [Cu(MeCN)$_4$]PF$_6$ (0.906 g, 2.44 mmol, 5 mol-%). The soln. was stirred at 45° for 2 h. After removal of the solvent, the residue was suspended in a sat. soln. of EDTA in H_2O (0.5M, 500 ml). The aq. soln. was extracted with CH_2Cl_2 (3 × 1000 ml), and the combined org. layers were washed with aq. sat. NaCl (1000 ml), dried (Na_2SO_4), and all volatiles were removed at reduced pressure. The remaining residue was purified by CC (SiO_2; AcOEt/hexane 2:3 → 3:2) to give Fmoc-Nup(G)-OH (38.5 g, 44.9 mmol, 92%). Colorless solid. R_f (AcOEt/hexane 3:2) 0.35. The NMR spectra indicated two conformers (*cis* and *trans* conformation around the carbamate bound) in a ratio of 1:2.1. ^{1}H-NMR (400 MHz, (D_6)DMSO): major conformer: 8.71 (*s*, 1 H of guanine); 7.99 (*s*, 1 H of triazole);

7.94 – 7.88 (*m*, 2 H of Fmoc); 7.77 – 7.65 (*m*, 2 H of Fmoc); 7.50 – 7.29 (*m*, 4 H of Fmoc, 5 arom. H); 5.61 (*s*, CH_2 of triazole); 5.24 – 5.14 (*m*, H–C_γ(Pro)); 4.36 – 4.08 (*m*, H–C_α(Pro), CH_2 of Fmoc, CH of Fmoc, H–C_β(Pro)); 3.76 – 3.64 (*m*, H–C_β(Pro)); 2.92 – 2.81 (*m*, H–C_δ(Pro)); 2.42 – 2.32 (*m*, H–C_δ(Pro)); 1.42 (*s*, 2 Me_3C); minor conformer: 8.32 (*s*, 1 H of guanine); 7.98 (*s*, 1 H of triazole); 7.94 – 7.88 (*m*, 2 H of Fmoc); 7.77 – 7.65 (*m*, 2 H of Fmoc); 7.50 – 7.29 (*m*, 4 H of Fmoc, 5 arom. H); 5.69 (*s*, CH_2 of triazole); 5.24 – 5.14 (*m*, H–C_γ(Pro)); 4.55 – 4.47 (*m*, H–C_α(Pro)); 4.13 – 4.40 (*m*, CH_2 of Fmoc, CH of Fmoc, H–C_β(Pro)); 3.76 – 3.85 (*m*, H–C_β(Pro)); 2.95 – 3.04 (*m*, H–C_δ(Pro)); 2.46 – 2.53 (*m*, H–C_δ(Pro)); 1.51 (*s*, 2 Me_3C). ^{13}C-NMR (101 MHz, (D_6)DMSO): major conformer: 163.3; 157.6; 151.8; 151.7; 149.4; 143.5; 141.7; 136.8; 129.5; 129.2; 128.9; 128.7; 128.2; 126.2; 123.8; 121.2; 111.9; 69.0; 67.8; 59.6; 57.7; 52.4; 51.8; 47.7; 35.2; 35.1; 28.4; minor conformer: 163.3; 157.6; 151.8; 151.7; 149.4; 143.5; 141.7; 136.8; 129.5; 129.2; 128.9; 128.7; 128.2; 126.18; 123.8; 121.2; 111.9; 69.0; 67.8; 58.4; 57.7; 52.4; 51.8; 47.6; 36.2; 36.1; 28.4. HR-ESI-MS: 858.3569 ($[M+H]^+$, $C_{45}H_{48}N_9O_9^+$; calc. 858.3575).

Solid-Phase Peptide Synthesis (SPPS). For SPPS, *Rink Amide-ChemMatrix*® resin from *Biotage* was used. For coupling of Nup monomers to solid support, a soln. of the appropriate Nup monomer (4 equiv.) and COMU [19] (4 equiv.) were each dissolved in DMF (0.6M), and EtN^iPr_2 (8 equiv., dissolved in NMP, 3M) was added to the suspension of the amino functionalized resin in DMF after 3 min of preactivation. The mixture was shaken for 60 min and washed with DMF (5×) and CH_2Cl_2 (5×). Couplings were monitored by the qual. *Kaiser* [24] and Chloranil [25] tests.

For Fmoc deprotection, a soln. of 40% piperidine in DMF was added to the resin, and the suspension was agitated for 3 min. After filtration, the procedure was repeated for 10 min. The resin was then washed with DMF (7×). For the final acetylation step of peptides Ac-$[Nup(T)]_6$-NH_2, Ac-$[Nup(C)]_6$-NH_2, Ac-$[Nup(A)]_6$-NH_2, Ac-$[Nup(G)]_6$-NH_2, and **18**, $Ac_2O/Et_3N/CH_2Cl_2$ 8 : 1 : 1 (*v*/*v*/*v*) was added to the resin. The mixture was agitated for 15 min and washed with CH_2Cl_2 (5×). For cleavage and deprotection of the peptides from solid support, the resin was agitated for 1 h in TFA/H_2O/TIS (=triisopropylsilane) 95 : 2.5 : 2.5 before collecting the soln. by filtration. This procedure was repeated twice, and the combined solns. were concentrated under reduced pressure. After addition of cold Et_2O, a white solid precipitated which was centrifuged, and the supernatant was decanted. The solid was suspended in Et_2O, sonicated, centrifuged, and the supernatant was decanted. The residual white solid was dissolved in H_2O and lyophilized to obtain white foam. For HPLC analyses and semiprep. purification, MeCN (*A*), and H_2O containing 1% of MeCN and 0.1% TFA (*B*) were used as eluents. The flow used for semiprep. HPLC was 6 and 1 ml/min for anal. HPLC. After semiprep. purification, all collected fractions were analyzed by anal. HPLC again, and only pure fractions were combined.

Data of Ac-[Nup(T)]₆-NH₂ (*=(4*S*)-1-Acetyl-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-proline*). HPLC: t_R 9.46 min; gradient: 90 to 40% *B* over 20 min at 50°. HR-ESI-MS: 1872.7261 ($[M+H]^+$, $C_{80}H_{90}N_{37}O_{19}^+$; calc. 1872.7214).

Data of Ac-[Nup(A)]₆-NH₂ (*=(4*S*)-1-Acetyl-4-{4-[(6-amino-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(6-amino-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(6-amino-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(6-amino-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(6-amino-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(6-amino-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-proline*). HPLC: t_R 5.59 min; gradient: 90 to 40% *B* over 20 min at 50°. HR-ESI-MS: 1926.7876 ($[M+H]^+$, $C_{80}H_{84}N_{55}O_7^+$; calc. 1926.7908).

Data of Ac-[Nup(C)]₆-NH₂ (*=(4*S*)-1-Acetyl-4-{4-[(4-amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(4-amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(4-amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(4-amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(4-amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}-*L*-prolyl-(4*S*)-4-{4-[(4-amino-2-oxopyrimidin-

*1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-proline*). HPLC: t_R 10.22 min; gradient: 98 to 70% *B* over 20 min at 50°. HR-ESI-MS: 1782.7246 ([*M* + H]$^+$, $C_{74}H_{84}N_{43}O_{13}^+$; calc. 1782.7234).

*Data of Ac-[Nup(G)]$_6$-NH$_2$ (=(4*S*)-1-Acetyl-4-{4-[(2-amino-6-hydroxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(2-amino-6-hydroxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(2-amino-6-hydroxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(2-amino-6-hydroxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(2-amino-6-hydroxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(2-amino-6-hydroxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-proline*). HPLC: t_R 11.58 min; gradient: 98 to 70% *B* over 20 min at 50°. HR-ESI-MS: 2022.7598 ([*M* + H]$^+$, $C_{80}H_{84}N_{55}O_{13}^+$; calc. 2022.7603).

*Data of (4*S*)-1-Acetyl-4-{4-[(2-amino-6-hydroxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(4-amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(6-amino-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(4-amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(2-amino-6-hydroxy-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl-(4*S*)-4-{4-[(6-amino-9*H*-purin-9-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-proline* (**18**). HPLC: t_R 11.17 min; gradient: 98 to 60% *B* over 20 min at 50°. HR-ESI-MS: 2203.8601 ([*M* + H]$^+$, $C_{91}H_{99}N_{54}O_{16}^+$; calc. 2203.8593).

*Data of N^6-[4-Carboxy-2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoyl]*-L*-lysyl*-L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl*-L*-prolyl*-L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl*-L*-prolyl*-L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl*-L*-prolyl*-L*-prolyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl*-L*-prolyl*-L*-lysinamide* (**19**). HPLC: t_R 13.70 min; gradient: 90 to 55% *B* over 20 min at 50°. HR-ESI-MS: 2617.1451 ([*M* + H]$^+$, $C_{125}H_{150}N_{37}O_{28}^+$; calc. 2617.1446).

*Data of N^6-[4-Carboxy-2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoyl]*-L*-lysyl*-L*-alanyl-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)*-L*-alanyl*-L*-alanyl*-L*-alanyl-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)*-L*-alanyl*-L*-alanyl*-L*-alanyl-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)*-L*-alanyl*-L*-alanyl*-L*-alanyl-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)*-L*-alanyl*-L*-alanyl*-L*-lysinamide* (**20**). HPLC: t_R 12.75 min; gradient: 90 to 55% *B* over 20 min at 50°. HR-ESI-MS: 1980.8254 ([*M* + H]$^+$, $C_{89}H_{114}N_{25}O_{28}^+$; calc. 1980.8260).

*Data of N^6-[4-Carboxy-2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoyl]*-L*-lysyl*-L*-alanyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl*-L*-alanyl*-L*-alanyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl*-L*-alanyl*-L*-alanyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl*-L*-alanyl*-L*-alanyl-(4*S*)-4-{4-[(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}*-L*-prolyl*-L*-alanyl*-L*-lysinamide* (**21**). HPLC: t_R 13.15 min; gradient: 90 to 55% *B* over 20 min at 50°. HR-ESI-MS: ($C_{109}H_{134}N_{37}O_{28}^+$; calc. [*M* + H]$^+$, 2409.0194). 2409.0202.

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Received October 5, 2012