Improved Large-Scale Liquid-Phase Synthesis and High-Temperature NMR Characterization of Short (F-)PNAs

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We report on a large-scale synthesis of F-PNA trimer **10** and PNA trimer **11**. The key improvement is the facile two-step synthesis of (2,4-difluoro-5-methylphenyl)acetic acid (**2**). Water solubility of the corresponding F-PNA oligomer **10** was achieved by synthesizing solubility enhancer **5a**, which is twofold positively charged and only consists of inherent structural elements of PNA. Protected and unpaired PNA *n*-mers exist in a mixture of 2^n conformers undergoing slow exchange and leading to complicated NMR spectra. Structure analysis was improved by recording ^{1}H- and ^{13}C-NMR spectra at elevated temperatures above the coalescence point. Fully protected backbone derivatives show sharp resonances where expected, and spectra of protected PNAs are remarkably simplified, thereby allowing an interpretation for the first time. Both trimers **10** and **11** are considered as building blocks for a self-replicating system based on PNA.

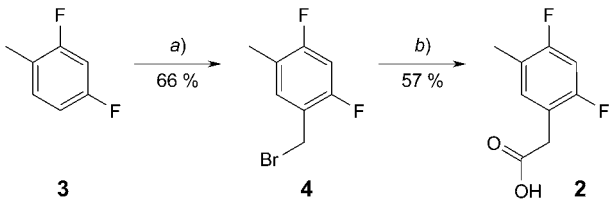
1. Introduction. – ^{19}F-NMR Probes have become a powerful tool for studying secondary-structure phenomena in RNA and DNA [1–10]. Yet, this method has not been utilized for analyzing PNA, a DNA or RNA mimic based on a non-charged, achiral, and pseudopeptidic backbone consisting of *N*-(2-aminoethyl)glycine (Aeg; **1**) units [11]. Moreover, there are only five reports on fluorine modified PNA: *i*) a multi-step synthesis of (2,4-difluoro-5-methylphenyl)acetic acid (**2**) and its Boc-protected monomer [12], *ii*) a hybridization survey of PNA containing fluoroaromatic residues [13], *iii*) two studies on synthesis and hybridization of fluorinated olefinic PNA [14][15], and *iv*) one on ^{18}F labeling [16].

PNA is reasoned as a model structure for a primordial genetic material [17–23] and was recently used in the design of '*protocells*' [24–27] that are not based on chemistry occurring in today's biosphere. While templated reactions involving PNA have been studied to a good extent [28–37], cases for an autocatalytic feedback have not been identified so far. Thus, we became interested in the potential of PNA to undergo self-replication and considered a kinetic NMR titration [38] assay as a useful tool for online monitoring of the system. Among the possible nuclei, ^{19}F appeared to be the most attractive because of its sensitivity (83% compared to ^{1}H), natural abundance of 100%, high chemical-shift dispersion and, most notably, its sensitivity to slight changes in its supramolecular environment. As an example of the latter, alterations in the hybridization of nucleic acids can induce changes in the chemical shift up to 1.5 ppm [1]. This encouraged us to explore whether it would be possible to prepare PNA with reasonable fluorine probes and good solubility at a scale required for NMR experiments.

2. Results and Discussion. – 2.1. *Fluorine Reporter Group*. The introduction of fluorine probes by means of modified bases is the most common approach, as it places the F-atom in direct spatial proximity to the supramolecular recognition side. We considered the fluoroaromatic 2,4-difluorotoluene (**3**), a nonpolar and almost perfect isostere of thymine [39][40], as the most promising candidate. To the best of our knowledge, there is no commercial source for (2,4-difluoro-5-methylphenyl)acetic acid (**2**), and only one report on a very laborious six step synthesis which made use of highly toxic reagents [12].

Here, we present a facile two-step synthesis of **2**, starting from commercially available 2,4-difluorotoluene (**3**; *Scheme 1*). First, **3** was treated with paraformaldehyde and anhydrous HBr in AcOH as reported previously for the bromomethylation of aromatic hydrocarbons [41]. To compensate for the deactivating influence of the F-substituents, we modified the conditions by adding $ZnBr_2$ as *Lewis* acid catalyst. The desired benzene derivative **4** was obtained in 66% yield after 4 h of reaction and subsequent silica-gel chromatography. Conversion to the corresponding *Grignard* reagent, followed by treatment with gaseous CO_2 gave **2** in 38% overall yield after chromatography. In addition, these conditions were also suitable to prepare (2,4-difluorophenyl)acetic acid, the isostere of uracil, from 1,3-difluorobenzene (22% overall yield; data not shown).

Scheme 1. *Synthesis of F-AcOH* **2**

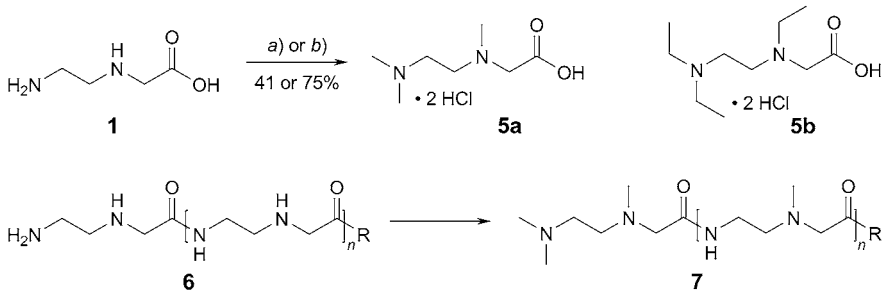


a) Paraformaldehyde (= polyoxymethylene), 33% HBr in AcOH, $ZnBr_2$, 120°, 4 h. *b*) 1. Mg, Et_2O, reflux; 2. CO_2, 0°, 1 h; 3. H^+, H_2O.

2.2. *Solubility Enhancer*. To overcome the drawbacks of the charge neutral backbone, namely reduced water solubility, pronounced self-organization [42], and limited cellular uptake [43], terminal positively charged modifiers such as the amino acid L-lysine [11] and the polyamine spermine [44], as well as backbone modifications [45–47] have been introduced. Furthermore, PNA/DNA chimeras [48][49], PNA-peptide conjugates [50][51], and PNA conjugates to high-molecular weight PEG [52] or polyethyleneimine [53] were developed. Typically, these methods involve the introduction of additional reaction sites and/or stereogenic centers inducing preferred helical orientations [42]. An improved strategy involved Fmoc-protected monomers bearing either positively charged or neutral solubility enhancers as side chains instead of nucleobases [54]. However, for N-terminal modification we envisioned a solubility enhancer to be as similar to the original PNA structure as possible. This was achieved by synthesizing a fully *N*-methylated backbone unit by *Leuckart–Wallach* chemistry or *via* reductive amination with elemental hydrogen, respectively (*Scheme 2*). The resulting achiral solubility enhancer **5a** is twofold positively charged at physiological

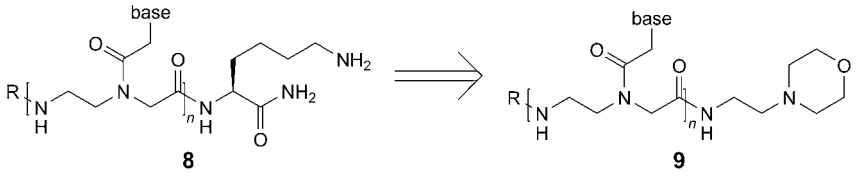
pH, while in accordance with the PNA structure. The same advantages also count for its ethyl analog **5b**. Additional charges can, in principle, be introduced by using oligomeric backbone molecules **6** as starting material for the preparation of polycharged modifiers **7**. Concerning the C-terminus, the common L-lysine amide **8** was replaced by *N*-(morpholinoethyl)amide **9**, thereby maintaining a positive charge and the achiral PNA structure (*Scheme 3*).

Scheme 2. *Synthesis of the Solubility Enhancer* **5a**, *Structure of the Ethyl Analog* **5b**, *and Approach to Higher Homologs* **7**



a) 1. Formalin solution, HCOOH, reflux, overnight; 2. HCl. *b*) 1. Formalin solution, H_2O, 0° → room temperature (r.t.), 1 h; 2. H_2, Pd/C.

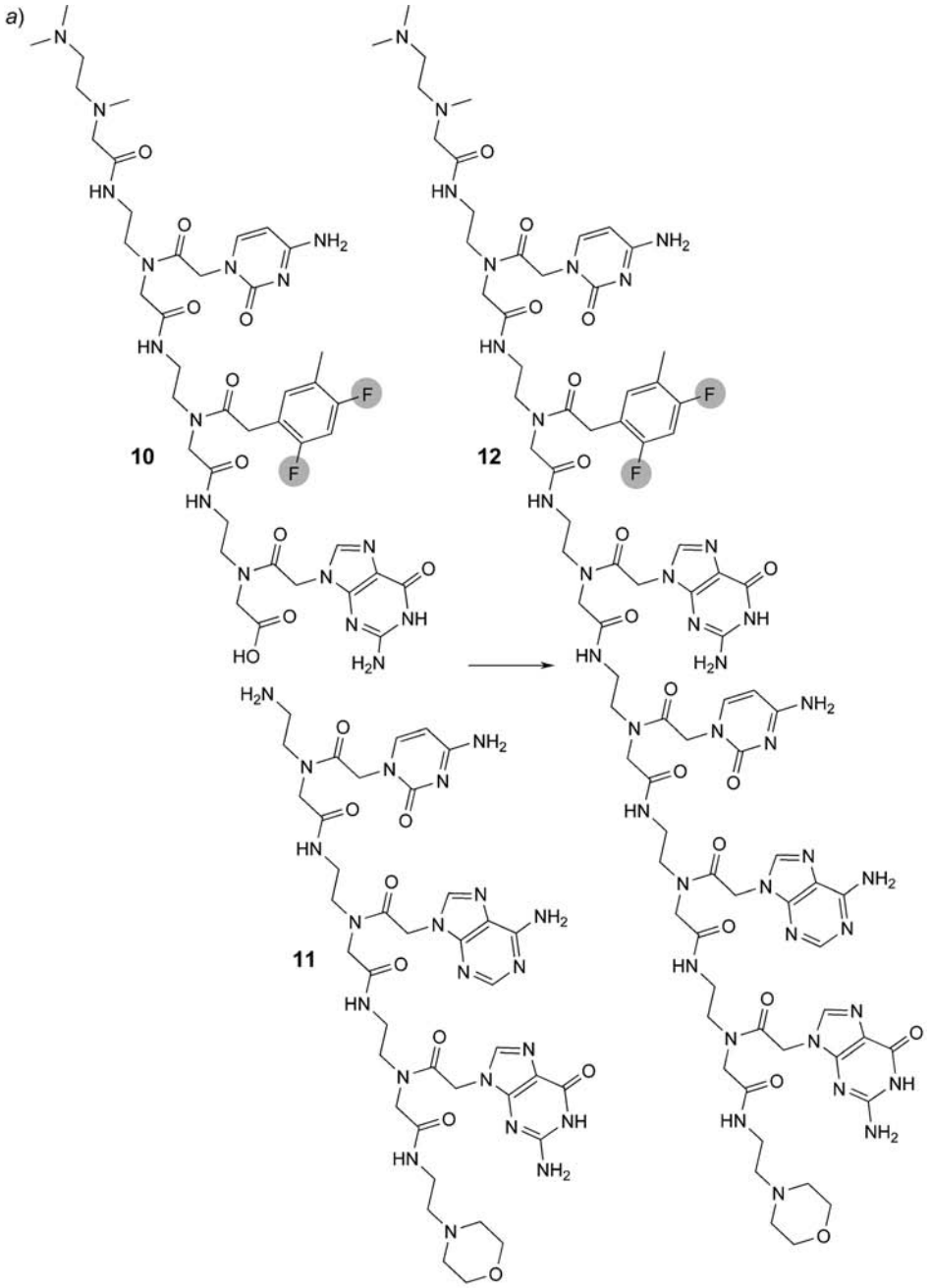
Scheme 3. *Widespread* C-*Terminal* L-*Lysine Amide* **8** *and Its Achiral Replacement* **9**



2.3. *Sequence Design*. We decided to work with a system of two trimeric building blocks leading to a hexa-PNA template with palindromic sequence upon condensation (*Scheme 4,a*). Thus, we would be able to compare this system with earlier studies from our laboratory [55–57]. The condensation would result in a central amide linkage and, for instance, be facilitated by the water-soluble EDC.

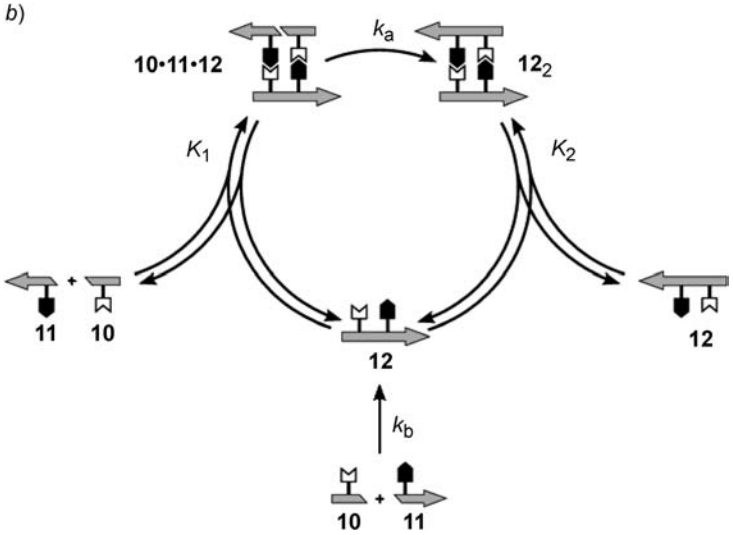
Starting point of the template design was the crystal structure of a self-complementary PNA (^{N}cgtacg^{C}) duplex [58]. As described above, natural thymine was replaced by 2,4-difluorotoluene (**3**), and the extremities were modified with the unreactive solubility enhancers **5a** and **9**. The sequence itself was slightly modified to place the isostere in the central position of the N-terminal trimer rather than on the ligation side (^{N}cfgcag^{C}). This position ensures minimal influence of the isostere on the ligation, while maintaining the fluorine probe in equal spatial proximity to the recognition side. In summary, we came up with a system consisting of building blocks F-PNA **10** and PNA **11**, which form, upon ligation, template F-PNA **12** (k_b; *Scheme 4,b*), which is assumed to be able to reversibly preorganize another pair of **10** and **11** (K_1) by H-bonding in a termolecular complex [**10**·**11**·**12**]. Thus, a pseudo-unimolecular

Scheme 4. *a*) *Trimeric Building Blocks* **10** *and* **11** *Give the Self-Complementary Hexa-PNA* **12** *upon Condensation. b*) *A Model of the Envisioned Self-Replicating System Based on PNA.*



Scheme 4 (cont.)

b)

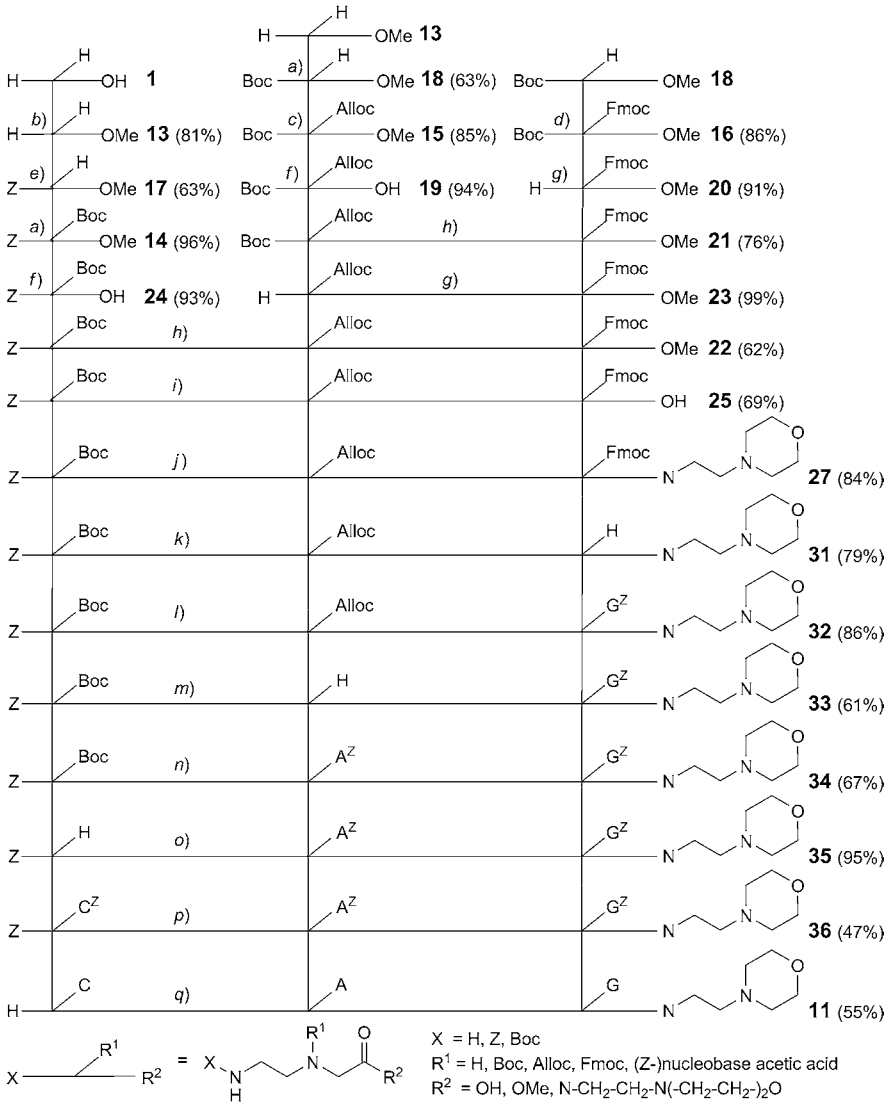


ligation reaction (k_a) should take place leading to template duplex $\mathbf{12_2}$. The reversible dissociation of this duplex affords two template molecules **12**, which may both enter another replication cycle. However, from thermodynamic considerations and data for published replication systems, it can be concluded that most template molecules **12** will be present as [**12**·**12**], a small fraction as [**10**·**11**·**12**], and another small portion in an unpaired state. Further information on this topic can be found in [59].

2.4. *(F-)PNA Synthesis.* PNA typically is synthesized in the solid phase from Fmoc/Bhoc [60], Boc/Z [61], Fmoc/Mmt [62], or Fmoc/Z [63] monomers according to peptide-synthesis protocols at a scale between 5 and 20 μmol. Strategies for a more economical large-scale synthesis of short PNAs in the liquid phase were extensively and almost exclusively elaborated by *Condom* and co-workers [64–71]. We adopted his most successful route, the 'fully protected backbone approach' (FPBA) [66], to prepare PNAs **10** and **11** on a large scale (*ca.* 150 μmol). This strategy requires the synthesis of a fully protected linear poly[*N*-(2-(aminoethyl)glycinamide) (poly-AEG) bearing as many different and orthogonal protecting groups on its secondary amines as there are different types of nucleic bases in the PNA sequence. Moreover, these protecting groups must be orthogonal to the protecting groups on the nucleobase acetic acid units. Additionally, care has to be taken of appropriate protection or modification of the elongation sites at the C- and N-termini.

The synthesis of PNA **11** is outlined in *Scheme 5*. It required 2-aminoethylglycine (**1**) that was synthesized as reported in [72] and subsequently transferred to its methyl ester **13**. The methyl ester **13** served as starting material for the three orthogonally protected key synthons **14**, **15**, and **16**. Treatment of **13** with *N*-[(benzyloxy)carbonyloxy]succinimide (→63% of **17**) and subsequent Boc protection of the remaining secondary amine gave the key synthon **14** in 60% overall yield. Another portion of **13** was protected at its primary amine upon reaction with Boc_2O (→63% of **18**) and

Scheme 5. *Synthesis of PNA* **11**

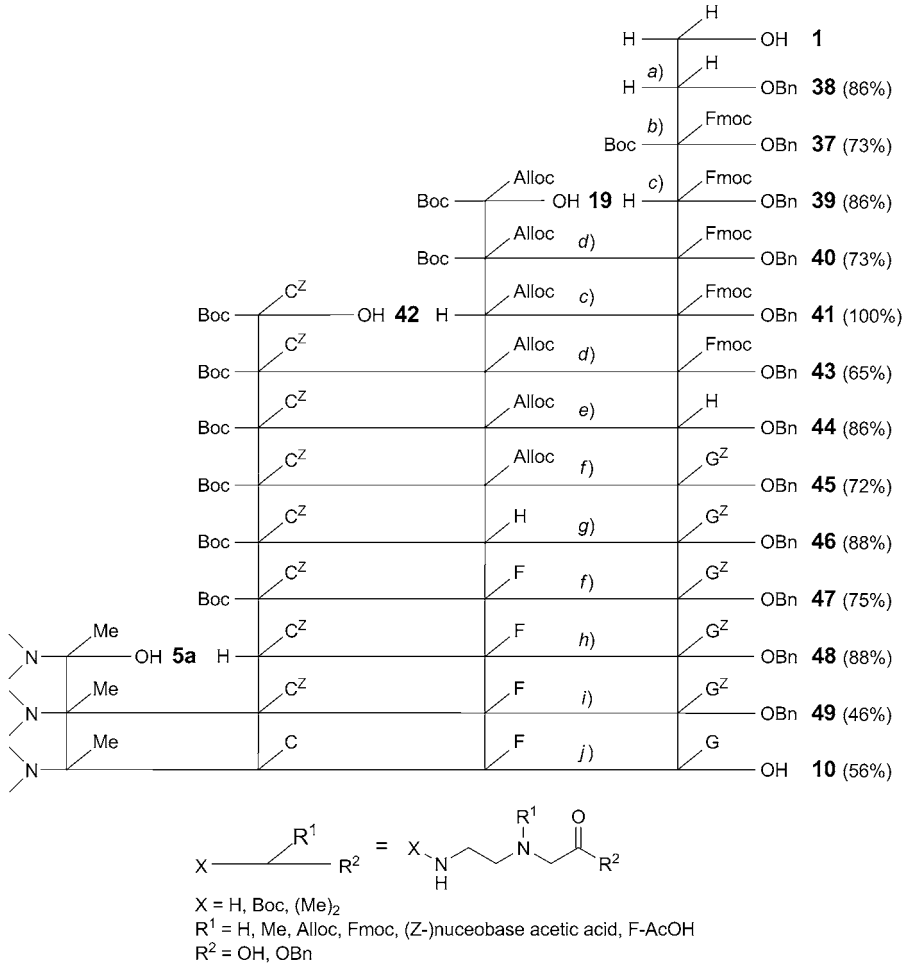


a) Boc_2O, Et_3N, CH_2Cl_2, $-10° \rightarrow$ r.t., overnight. *b*) $SOCl_2$, MeOH, reflux, overnight. *c*) Allyloxycarbonyl chloride (Alloc-Cl), Et_3N, CH_2Cl_2, $0° \rightarrow$ r.t., 2 h. *d*) [(9*H*-Fluoren-9-yl)methoxy]carbonyl chloride (Fmoc-Cl), EtN^iPr_2, CH_2Cl_2, $0° \rightarrow$ r.t., 4 h. *e*) *N*-[(Benzyloxy)carbonyloxy]succinimide (Z-OSu), *N*-methylmorpholine (NMM), MeCN, $-15° \rightarrow$ r.t., 5 h. *f*) 1M LiOH, THF, $0° \rightarrow$ r.t., 2 h. *g*) TFA, CH_2Cl_2, $0° \rightarrow$ r.t., 2 h. *h*) 2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), EtN^iPr_2, DMF, r.t., overnight. *i*) 1. 1M LiOH, THF, $0° \rightarrow$ r.t., 2 h; 2. Fmoc-Cl, 0°, 1 h. *j*) 1. *N*,*N*′-Dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (HOSu), CH_2Cl_2, $0° \rightarrow$ r.t., overnight; 2. 2-morpholinoethylamine (**26**), $-15° \rightarrow$ r.t., 4 h. *k*) Et_2NH, CH_2Cl_2, r.t., 1.5 h. *l*) G^ZCH_2COOH (**28**), Bromotris(dimethylamino)phosphonium hexafluorophosphate (Brop), Et_3N, CH_2Cl_2, $0° \rightarrow$ r.t., overnight. *m*) $Pd[P(Ph)_3]_4$, Et_2NH, CH_2Cl_2, $0° \rightarrow$ r.t., 1 h. *n*) A^ZCH_2COOH (**29**), Brop, Et_3N, DMF, $0° \rightarrow$ r.t., overnight. *o*) TFA, Et_3SiH, CH_2Cl_2, $0° \rightarrow$ r.t., 2 h. *p*) C^ZCH_2COOH (**30**), Brop, Et_3N, DMF, $0° \rightarrow$ r.t., overnight. *q*) Trifluoromethanesulfonic acid, TFA, *m*-cresol, thioanisole, r.t., 2 h.

subsequently treated with Alloc-Cl or Fmoc-Cl to give the synthons **15** and **16**, respectively. Prior to the following condensation steps, **14** and **15** were hydrolyzed with 1M LiOH, and the Boc group of **16** was removed by acidolysis with CF_3COOH (TFA)/CH_2Cl_2 1:1. Coupling of fragments **19** and **20** was carried out by HBTU activation to afford the desired bis-AEG **21** in 76% yield after purification by silica-gel chromatography. Subsequent Boc removal enabled elongation to tris-AEG **22** by condensation of the resulting amine **23** with carboxylic acid **24**. The fully protected backbone **22** was obtained in 62% yield after silica-gel chromatography. The following ester hydrolysis resulted in the simultaneous removal of the Fmoc group which was easily reversed by addition of Fmoc chloride after complete saponification (→69% of **25**). Next, the solubility enhancer was introduced *via* pre-activation of **25** with DCC and HOSu in CH_2Cl_2, followed by addition of 2-morpholinoethylamine (**26**) to afford compound **27** in 84% yield. The remaining steps consisted of three deprotection–coupling cycles to introduce the nucleobase moieties in form of their Z-protected acetic acids **28**–**30**. These compounds were synthesized according to slightly modified procedures [60][73]. Selective removal of the Fmoc group on **27** (79% yield), by means of Et_2NH in CH_2Cl_2, was followed by condensation of guanine unit **28** to **31** with Brop in $CHCl_3$. The desired compound **32** could be isolated in 86% yield after silica-gel chromatography. Treatment of **32** with $Pd[P(Ph)_3]_4$ and Et_2NH as the allyl scavenger gave amine **33** after 30 min. Subsequent attachment of adenine unit **29** to **33** was also mediated by Brop reagent, but carried out in DMF instead of $CHCl_3$, as the solubility of the growing PNA decreased. Purification of the crude product by step-gradient chromatography on silica gel required highly polar conditions and gave the target compound **34** in 67% yield. Removal of the remaining Boc group led to the corresponding TFA salt **35** in 95% yield after precipitation with cold Et_2O. Conjugation of the *N*-Z cytosine unit **30** with **35** by means of Brop activation afforded the fully protected tri-PNA **36** in 47% yield after purification by semi-preparative RP-HPLC. The coupling efficiency dropped by 20% after each introduction of an additional nucleobase unit, accompanied by reduced solubility of the corresponding oligomers. The synthesis of the target PNA **11** was achieved by submitting **36** to TFMSA–TFA containing thioanisole and *m*-cresol as scavengers, which induced simultaneous deprotection of both the nucleobases and the N-terminal ligation side. The crude product was precipitated with cold Et_2O, and the residue was purified by semi-preparative RP-HPLC to give **11** in 55% yield after lyophilization. Its purity and structure was confirmed by RP-HPLC analysis, ^{1}H-NMR spectroscopy, MALDI-TOF, and high-resolution ESI mass spectrometry.

For circumventing chromatographic problems likely to occur in the presence of the highly polar, twofold charged solubility enhancer **5a**, tri-PNA **10** was synthesized according to a plan in which this moiety is introduced at an advanced step of the synthesis. Following the FPBA strategy, this would necessitate the implementation of another orthogonal protective group. To overcome this, a mixed strategy combining PNA units as well as fully *N*-protected *N*-(2-aminoethyl)glycinamide synthons was chosen [70] (*Scheme 6*). To this end, synthon **37** was prepared from **1** by acid-catalyzed esterification with BnOH under H_2O-removing conditions (*Dean–Stark* trap; 86%, **38**), protection of the primary amine with the Boc group, followed by masking the secondary amine with the orthogonal Fmoc group. We improved the overall yield for **37**

Scheme 6. *Synthesis of PNA* **10**



a) BnOH, TsOH, toluene, 140°, Ar, overnight. *b*) 1. Boc_2O, Et_3N, CH_2Cl_2, 0° → r.t., 5 h; 2. Fmoc-Cl, EtN^iPr_2, CH_2Cl_2, 0° → r.t., overnight. *c*) TFA, CH_2Cl_2, 0° → r.t., 2 h. *d*) HBTU, EtN^iPr_2, DMF, r.t., overnight. *e*) Et_2NH, CH_2Cl_2, r.t., 1.5 h. *f*) G^ZCH_2COOH (**28**) or FCH_2COOH (**2**), Brop, Et_3N, DMF, 0° → r.t., overnight. *g*) $Pd[P(Ph)_3]_4$, *N*,*N*′-dimethylbarbituric acid (NDMBA), THF, 0° → r.t., 2 h. *h*) TFA, Et_3SiH, CH_2Cl_2, 0° → r.t., 2 h. *i*) 1. DCC, HOSu, CH_2Cl_2, 0° → r.t., overnight; 2. **5**, −15° → r.t., 5 h. *j*) TFMSA, TFA, *m*-cresol, thioanisole, r.t., 2 h.

by introducing both carbamate moieties sequentially in a one-pot reaction (73 *vs.* 41% overall yield). *N*-Deprotection of **37**, followed by HBTU-mediated coupling of the resulting fragment **39** with **19**, led to bis-AEG **40**. After acidolysis of the Boc group of **40** (→ quant. yield of **41**), condensation with Z-cytosine PNA fragment **42** was carried out *via* another HBTU coupling. Deprotection of the Fmoc group from **43** and subsequent introduction of G^ZCH_2COOH **28** into **44** were carried out as described for

compounds **31** and **32**, respectively. Unfortunately, Pd-catalyzed Alloc deprotection of **45** in the presence of Et_2NH proceeded in low yield. Allylamine formation is considered as the most prominent side reaction, since the deprotected amine competes with the scavenger in the trapping of the π-palladium complex [74]. Additionally, it is known that secondary amines like Et_2NH are protonic reversible allyl-group trapping reagents that accessorily promote allylamine formation through an equilibrium process. Examples of alternative and irreversible scavengers include CH-acidic compounds like dimedone or *N*,*N*-dimethylbarbituratic acid (NDMBA). When the latter one was used, the cleavage proceeded selectively and smoothly to give the desired amine **46** in 88% yield. Subsequent implementation of the fluorine probe was achieved by coupling FCH_2COOH (**2**) by means of Brop reagent. After TFA treatment of the resulting tri-PNA **47**, the solubility enhancer **5a** was introduced *via* preactivation with DCC and HOSu. Due to its highly polar character, the crude product was purified by semi-preparative RP-HPLC to afford **49** in 46% yield. Finally, the water-soluble target PNA **10** was obtained by submitting **49** to cleavage with TFMSA and TFA, as described for the preparation of PNA **11** (56% yield). After purification by RP-HPLC, its purity and structure could be verified (RP-HPLC, ^{1}H-NMR, ^{19}F-NMR, MALDI-TOF-MS, HR-ESI-MS). Analytical RP-HPLC plots and MALDI-TOF mass spectra of tri-PNAs **10** and **11** are depicted in *Fig. 1*.

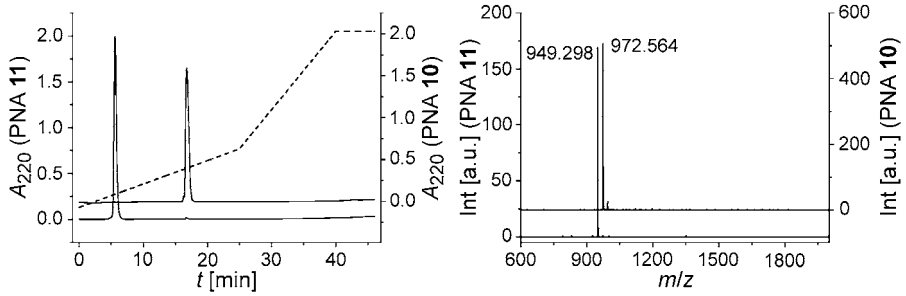
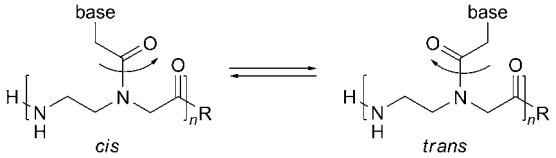


Fig. 1. *RP-HPLC Traces* (left) *and MALDI-TOF-MS spectra* (right) *of* **10** *and* **11**

2.5. *NMR Spectroscopy.* In complexes of PNA with DNA [75][76], RNA [77], and itself [58], the CO group of the backbone-base linker points exclusively to the C-terminus (*cis*-rotamer), whereas another rotameric form (*trans*-rotamer) coexists in ssPNA and monomers (*Scheme 7*). Moreover, this effect also occurs if the secondary amine of the backbone is protected with a carbamate moiety. The activation energy for the interconversion has been determined as 19 ± 2 kcal/mol by variable-temperature ^{1}H-NMR spectroscopy on four PNA monomers and one dimer [78]. The rate of

Scheme 7. cis- *and* trans-*Rotamers of a PNA Unit*



exchange was calculated as 0.5 – 2 s^{-1} at 37°. Hence, protected and unpaired PNA *n*-mers exist in a mixture of 2^n conformers in slow exchange and generate complicated NMR spectra. This complication can only be avoided by the design of suitable PNA analogs where conformational constraints exclude the formation of rotational isomers [79 – 82].

Since we needed a detailed analysis of our poly-AEGs and poly-PNAs, we decided to accelerate the *cis* ⇌ *trans* equilibria by elevating the temperature. ^{1}H-NMR Spectra of tris-AEG **22** at different temperatures revealed a profile typical for the determination of rotational barriers (*Fig. 2*). At low temperatures, each H-atom displayed several resonances due to the putative presence of eight conformers of **22** which could not be differentiated. Then, in an intermediate temperature range (40 – 70°), the spectrum consisted of significantly broadened overlapping lines which coalescenced upon further heating to give single, flat-topped peaks. Finally, in the regime after coalescence (70 – 120°), the exchange was fast, and sharp lines could be observed. The data suggest that the barrier heights and, therefore, the coalescence temperatures (T_c) associated with the given protective groups differ in accordance with their steric demand (Fmoc ≫ Alloc and Boc), and depend on their conformational flexibility that arises from their position within the sequence (Alloc > Boc). However, an improved ^{1}H resonance assignment of **22** was achieved at 100°. As a single AEG backbone unit contains four geminal H-atom pairs with nearly isochronous chemical shifts, a complete assignment in this region was not possible. The ^{13}C-NMR spectra

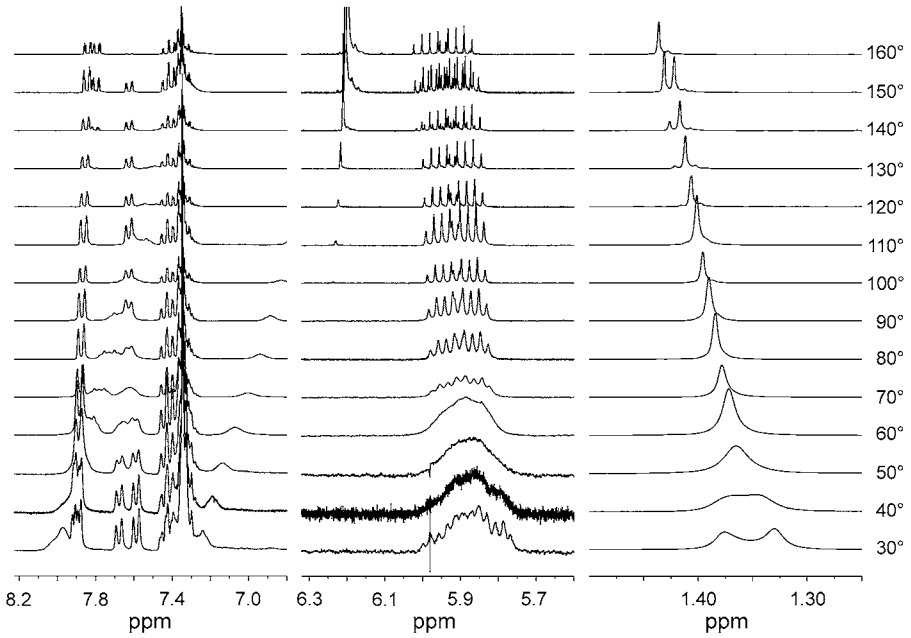


Fig. 2. *Cutouts of ^{1}H-NMR spectra* (250 MHz, (D_6)DMSO) *of Z-Aeg(Boc)-Aeg(Alloc)-Aeg(Fmoc)-OMe* (**22**) *at different temperatures.* Resonances assigned to aromatic CH of Fmoc and amide NH groups (*left*), AllocCH_2C*H*=CH_2 (*middle*), and Me group of Boc (*right*).

benefit notably from increased signal-to-noise ratio as illustrated in *Fig. 3*. Given that conformers are populated according to their relative free energies, one can accomplish a more than eightfold saving of time or substance.

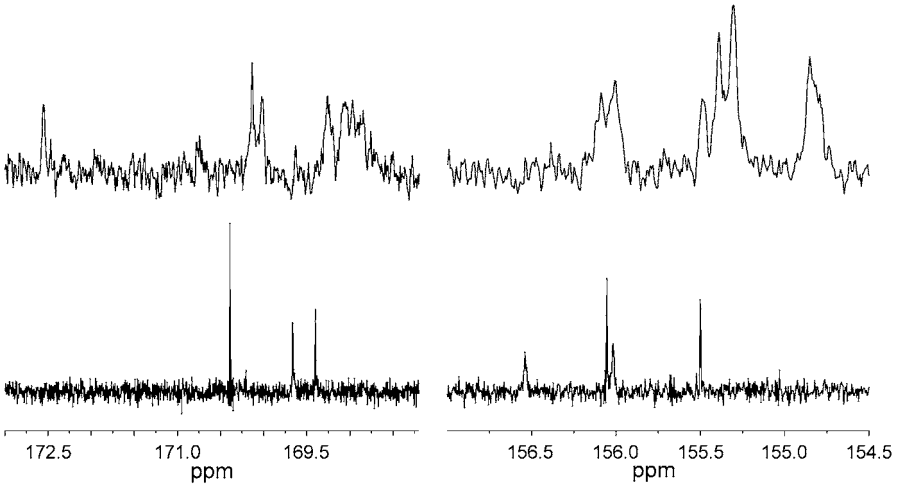


Fig. 3. *Low-field region of* ^{13}*C-NMR spectra of Z-Aeg(Boc)-Aeg(Alloc)-Aeg(Fmoc)-OMe* (**22**) *at 30°* (100 MHz, 10835 scans; *above*) *and 100°* (63 MHz, 1024 scans; *below*). Resonances assigned to 3 CO of Gly (*left*) and 4 carbamate-CO (*right*).

Boc-Aeg(C^Z)-Aeg(Alloc)-Aeg(H)-OBn (**44**) is an example for a class of hybrid molecules, obtained on the way from the fully protected tris-AEGs *via* the fully protected tri-PNAs to the unprotected tri-PNAs. One of its secondary amines is free, one is protected, and the third one is already modified with a protected nucleobase. As a consequence of the relative low steric demand of only two substituents, the temperature-dependent ^{1}H-NMR spectra of **44** showed that rotational barriers were crossed frequently at 90° to obtain single signals for each H-atom (*Fig. 4*).

In accordance with the challenges during synthesis and purification, every additional nucleobase unit complicated the analysis by raising the coalescence temperature. Boc-Aeg(C^Z)-Aeg(Alloc)-Aeg(Fmoc)-OBn (**43**), for instance, showed a single set of ^{1}H signals at 110° and 200 MHz, while the spectrum of the G^Z-modified derivative Boc-Aeg(C^Z)-Aeg(Alloc)-Aeg(G^Z)-OBn (**45**) exhibited broader lines and multiple signals consistent with at least three rotamers (*Fig. 5*). Nonetheless, compared with the spectrum at 30°, a significant progress in structural analysis could be achieved by reducing line broadening and the number of resonances for chemical equivalent nuclei.

The ^{1}H-NMR spectra of the fully protected tri-PNA **49** were recorded in a temperature range of 30–150° (*Fig. 6*). The signals assigned to the FCH_2 group (2.0–2.2 ppm) showed coalescence at 70° and finally gave rise to one defined signal at 90°. In contrast, the resonances assigned to C^Z-C(5)H (6.9 ppm) and C^Z-C(6)H (7.8 ppm) groups had a T_c value of 80°. Decomposition of the molecule started slowly at 110° and could be followed by the appearance of a resonance at 4.5 ppm. It is noteworthy that

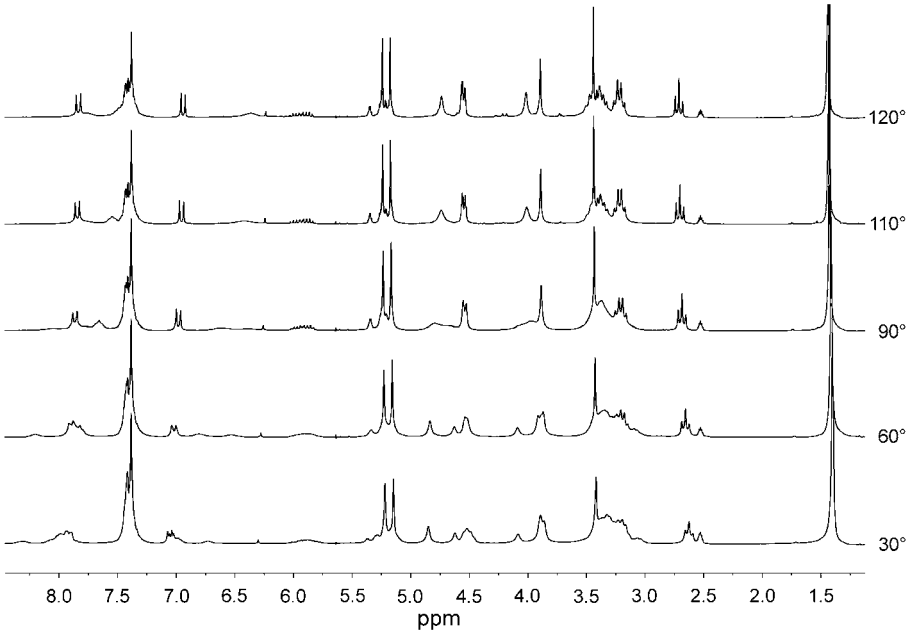


Fig. 4. *^{1}H-NMR Spectra* (200 MHz, (D_6)DMSO) *of Boc-Aeg(C^Z)-Aeg(Alloc)-Aeg(H)-OBn* (**44**) *at different temperatures*

signals assigned to the *N*-methyl and the *N*,*N*-dimethyl group of the solubility enhancer revealed different values for T_c. The latter ones (2.8–2.9 ppm) showed coalescence at 60° and exhibited a sharp signal at 70°, while the broad *N*-Me signals demonstrated pronounced shift-shifting (2.7 → 2.4 ppm), and reached the coalescence point and the regime of fast exchange after an additional temperature raise of 20°. This example indicates that the distance to the nearest rotation center, as well as the overall conformal flexibility, have a strong influence on the shift dispersion and on T_c of a given nucleus. Of course, distance and conformal flexibility are connected with each other. Anyway, we were able to analyze the spectrum at 110° by integrating non-overlapping as well as overlapping signals, and assigning resonances to single H-atoms or classes of similar ones (*Fig. 7*).

Spectra of the corresponding unprotected tri-PNA **10**, recorded between 30–140°, did not show a single set of resonances until 100°, while narrow line-widths for all C-bound H-atoms before 140° (*Figs. 8* and *9*). A broadened signal at 6.2 ppm was detected for diverse protonated NH species and the COOH group.

In contrast, tri-PNA **11** (*Fig. 10*) and its fully protected derivative **36** (not shown) revealed multiple datasets and line broadening under the above conditions and while decomposition upon further heating. Both compounds differ from the preceding ones with respect to their end modifications and their central nucleobase (adenine instead of 2,4-difluorotoluene). Hence, we assume that the higher values for T_c are associated with the higher steric demand of (protected) adenine compared to the fluoroaromate.

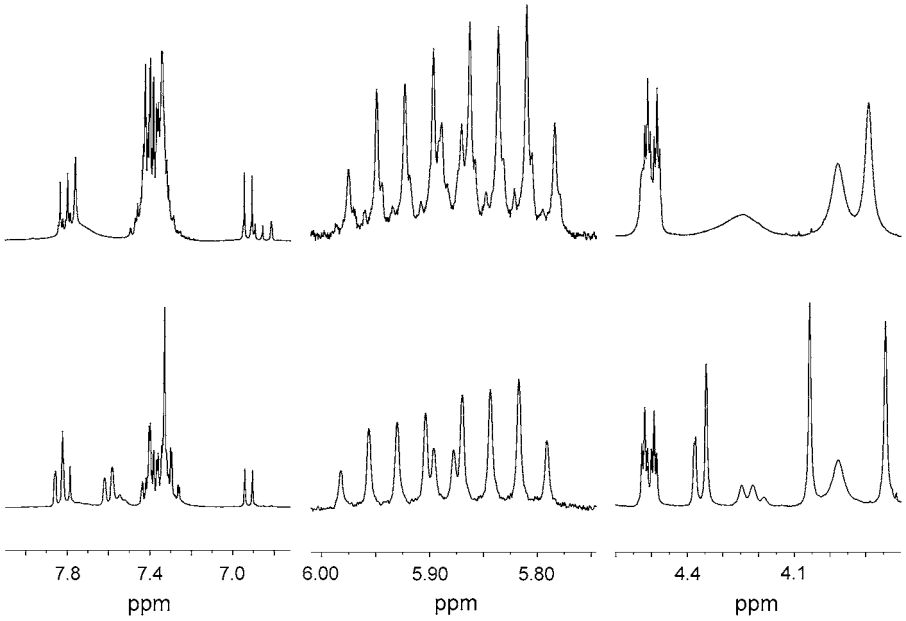


Fig. 5. *Expansions of* ^{1}*H-NMR spectra* (200 MHz, (D_6)DMSO, 110°) *of Boc-Aeg(C^Z)-Aeg(Alloc)-Aeg(Fmoc)-OBn* (**43**; *below*) *and Boc-Aeg(C^Z)-Aeg(Alloc)-Aeg(G^Z)-OBn* (**45**; *above*). Resonances assigned to the nucleobase(s), the aromatic H-atoms, and the amide NH (*left*), AllocCH_2C*H*=CH_2 (*middle*), and the CH_2 groups, including CH groups of Fmoc (*right*).

3. Conclusions and Outlook. – In summary, we designed a system to evaluate potential PNA self-replication by kinetic ^{19}F-NMR titration and presented an efficient large-scale synthesis of the building blocks needed. In particular, an improved synthesis of (2,4-difluoro-5-methylphenyl)acetic acid (**2**) was presented, reducing the number of required steps from six to two. Furthermore, the development of an achiral N-terminal solubility enhancer has been an essential point of our survey. This was addressed with the synthesis of the fully *N*-methylated and twofold charged backbone **5a** that was designed to be as similar to the native PNA structure as possible. Moreover, we adopted and modified the 'fully protected backbone approach' (FPBA) of *Condom* and co-workers to prepare tri-PNAs **10** and **11**. Finally, we presented a solution to a hitherto ignored problem in the context of liquid-phase PNA synthesis, namely the complexity of NMR spectra due to the coexistence of 2^n conformers in slow exchange. To this end, 22 monomers, dimers, and trimers were studied by ^{1}H-NMR spectroscopy at elevated temperatures up to 160°. We could obtain spectra in the regime of overall fast exchange, showing a single set of signals for ten of these compounds. For further nine oligomers, spectra with a remarkably reduced number of resonances, as well as significantly narrowed line-widths, were acquired. Thus, a reasonable interpretation and assignment was able for the first time. Unfortunately, two compounds decomposed under the conditions studied, and in one case elevated temperatures led to a complication of the spectrum. Decomposition is especially a concern if the analyte contains unprotected

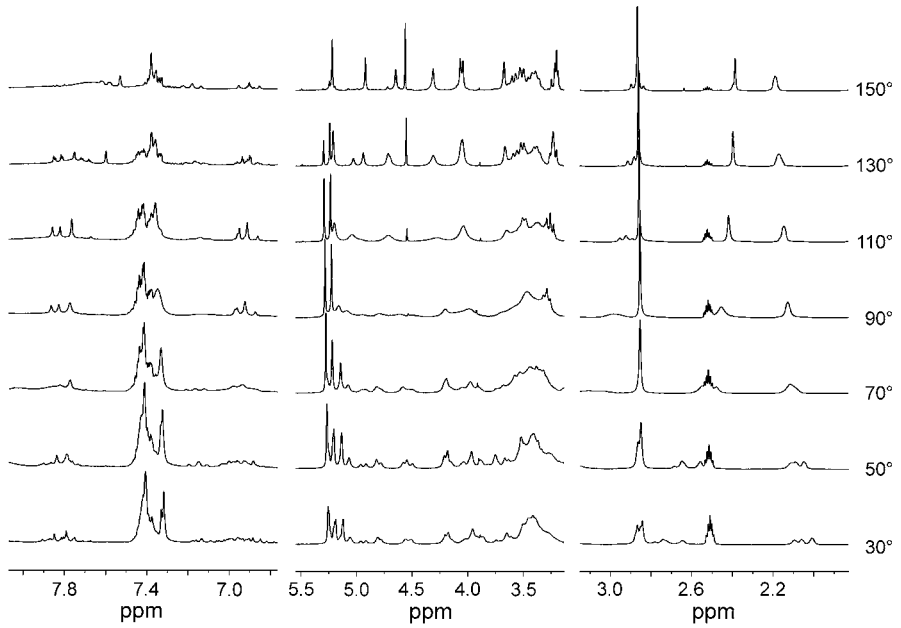


Fig. 6. *Expansions of* ^{1}*H-NMR spectra* (200 MHz, (D_6)DMSO) *of* **49** *at different temperatures.* Resonances assigned to the nucleobases, the aromatic H-atoms of the Ph rings, and amide NH (*left*), CH_2 (*middle*), and the Me groups, including one CH_2 signal shifting at 90° (*right*).

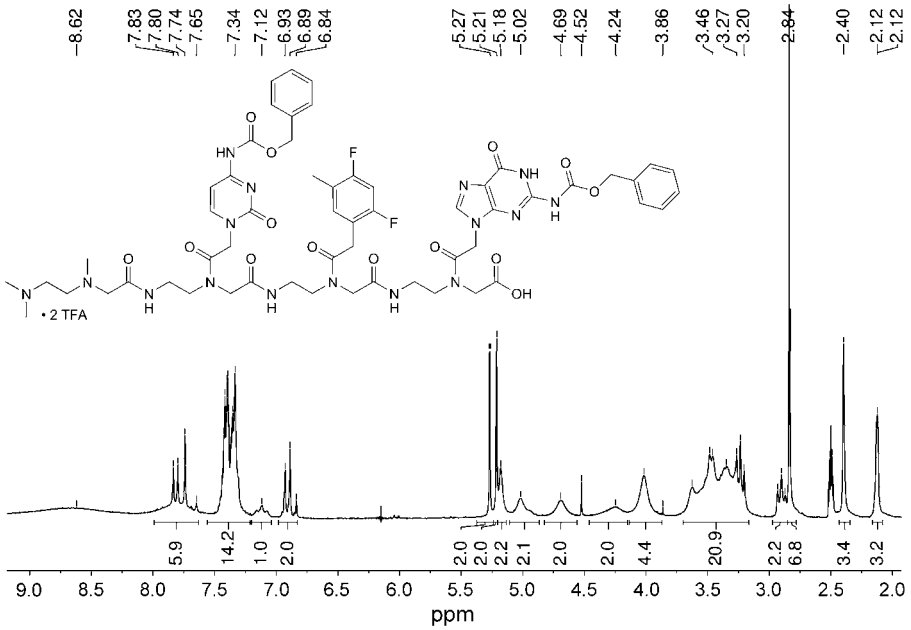


Fig. 7. ^{1}*H-NMR Spectrum* (200 MHz, (D_6)DMSO, 110°) *of* **49**. For assignments, see *Exper. Part.*

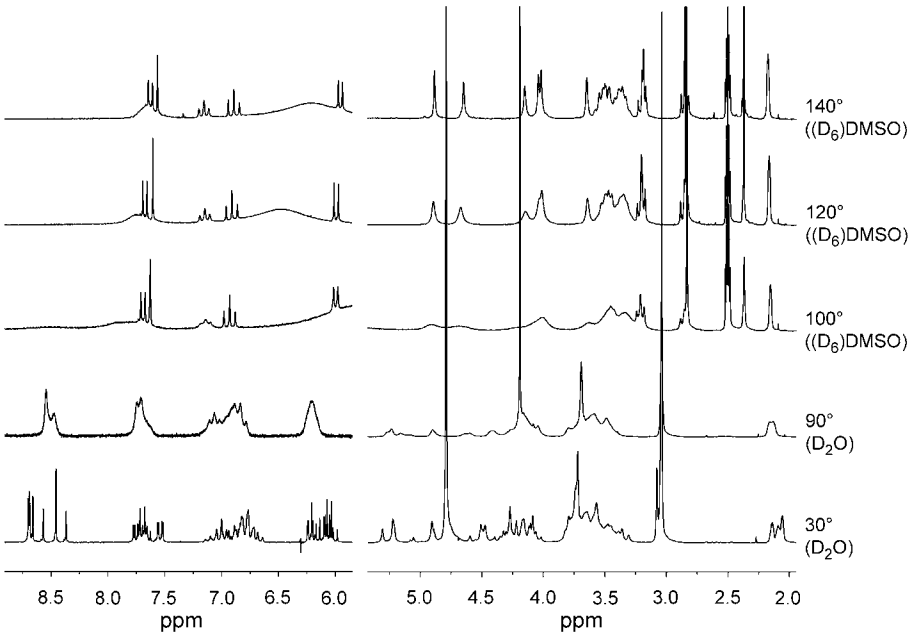


Fig. 8. *Expansions of* ^{1}*H-NMR spectra* (200 MHz, D_2O or (D_6)DMSO) *of tri-PNA* **10** *at different temperatures.* Resonances assigned to the nucleobases (*left*) and the remaining H-atoms (*right*). For details on calibration, see *Exper. Part*.

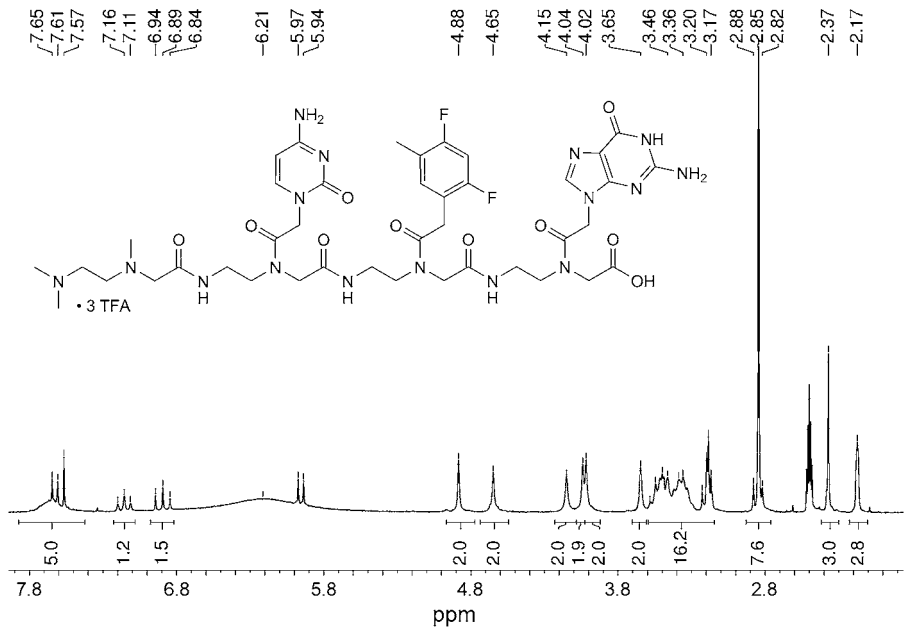


Fig. 9. ^{1}*H-NMR Spectrum* (200 MHz, (D_6)DMSO, 140°) *of* **10**. For assignments, see *Exper. Part.*

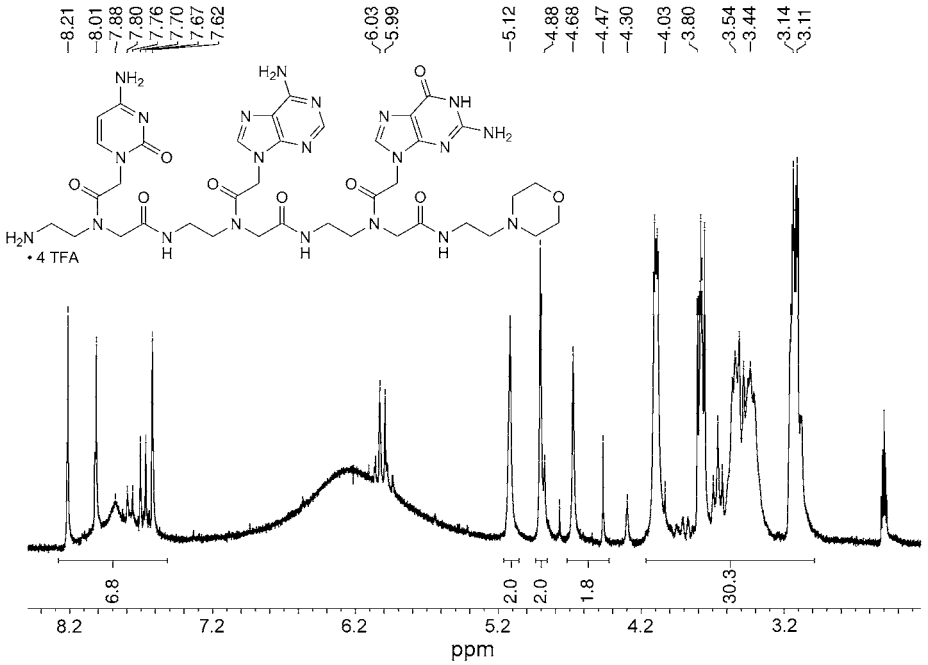


Fig. 10. *^{1}H-NMR Spectrum* (200 MHz, (D_6)DMSO, 140°) *of* **11**. For assignments, see *Exper. Part.*

reactive groups, *e.g.*, amines or carboxylic acids. Ester **22**, for instance, was stable enough to be analyzed by ^{13}C-NMR spectroscopy at 100°, while the corresponding carboxylic acid **25** decomposed under Fmoc elimination within a few minutes under these conditions. Clearly, high-temperature NMR characterization is also limited by the thermal stability of the spectrometer, especially the probe head. Beside this drawback, we would like to stress the importance of high-temperature NMR spectra for the success of our synthetic work presented here, as they provided unprecedented structural data of intermediate and desired compounds. Most notably, the spectra offer insight into the dynamic behavior of protected PNAs and AEGs for the first time. Altogether, the results provide the synthetic base for a large survey aiming at potential PNA self-replication. The search for optimal conditions for the autocatalytic template-directed ligation of both trimers is the subject of ongoing experimental studies in our laboratory. First results reveal that self-replication is detectable and controllable in novel ways. Full details of self-replication studies will be reported in due course.

Experimental Part

General. Nomenclature. Following the IUPAC nomenclature rules leads to complicated names (given in parentheses), especially for the larger molecules presented in this work. Therefore, we used a notation according to the one used for the description of peptide sequences. The diaminoacid *N*-(2-aminoethyl)glycine (Aeg; **1**) is the constitutional building block which is linked with further Aeg units, suitably protected or modified on its N-terminus (left end) or C-terminus. The substituents on the

internal secondary amine are indicated in parentheses as side-chain protective groups. For instance, unprotected Aeg **1** is denoted as H-Aeg(H)-OH.

Abbreviations. A: adenine, AcOH: acetic acid, Aeg: *N*-(2-aminoethyl)glycine, Aem: 2-morpholinoethylamine, Alloc: (allyloxy)carbonyl, Bhoc: (benzhydryloxy)carbonyl, Bn: benzyl, Boc: (*tert*-butyloxy)carbonyl, Brop: bromotris(dimethylamino)phosphonium hexafluorophosphate, C: cytosine, DCC: *N*,*N*′-dicyclohexylcarbodiimide, EDC: 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, F: 2,4-difluorotoluene, Fmoc: [(9*H*-fluoren-9-yl)methoxy]carbonyl, G: guanine, Gly: glycine, HBTU: 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronoium hexafluorphosphate, HOSu: *N*-hydroxysuccinimide, MALDI-TOF: matrix-assisted laser-desorption ionization time of flight, Mmt: 4-monomethoxytrityl, NDMBA: *N*,*N*′-dimethylbarbituric acid, NMM: *N*-methylmorpholine, TEA: triethylamine, TFA: trifluoroacetic acid, TFMSA: trifluoromethanesulfonic acid, Z: (benzyloxy)carbonyl.

All chemicals were used as delivered, usually in *p.a.* quality. All moisture-sensitive reactions were carried out under Ar. Dry DMF and CH_2Cl_2 were purchased from *Biosolve* or *Roth*. TLC: *Merck* silica gel *60 F-254* aluminum plates, visualization by inspection under UV light (254 nm) or by the use of phosphomolybdic acid stain (4.00 g phosphomolybdic acid hydrate in 100 ml MeOH). Column chromatography (CC): silica gel (SiO_2; *ICN* silica *32-63* 60 Å). Freeze drying: *Christ Alpha 1-2*. HPLC: *Applied Biosystems* Vision Workstation with *AFC2000 Roboter* and *Jetstream 2 Plus Column-Thermostat*; *A:* 0.1% TFA in deionized and dist. H_2O, *B:* 0.1% TFA in MeCN; T_{column}, 55°. Anal. HPLC: 250 × 4.6 mm *Supelco Ascentis RP-Amide* 5 μm; flow, 1 ml/min. Semi-prep. HPLC: 250 × 10 mm *Supelco Discovery BIO Wide Pore C18* 5 μm; flow, 3 ml/min. NMR: *Bruker DPX 200* (200 MHz), *DRX 250*, or *DRX 400* (400 MHz); δ in ppm rel. to TMS as internal standard and to external standard TFA for ^{19}F; *J* in Hz. Temp.-dependent spectra of unprotected PNAs **10** and **11**: since the chemical shift of the remaining undeuterated portions of the solvent is considerably more temp.-dependent in the case of D_2O than (D_6)DMSO, calibration was carried out as follows. The spectrum at 30° was calibrated to the solvent as internal standard. Afterwards, a distinct signal was chosen for calibration of the following spectra (**10**: *N*-Me group; **11**: MeCN, remaining from preceding HPLC purification). MS: *VG Instruments Autospec* (EI, FAB), *Thermo Scientific LTQ-Orbitrap XL* (HR-ESI), *Bruker daltonics autoflex* (MALDI-TOF; matrices: 2,5-dihydroxybenzoic acid, α-cyano-4-hydroxycinnamic acid, and 2′,4′,6′-trihydroxyacetophenone); only characteristic fragments are given with intensities [%] and possible composition in parentheses.

General Procedure for Removing the Boc Group (*GP 1*). The protected compound was placed in CH_2Cl_2 at 0°. In the presence of nucleobases, Et_3SiH was added as a scavenger. An equal amount of TFA was added. After stirring at 0° for 1 h and additional 2 h at r.t., the volatiles were removed by repeated co-evaporation with toluene. The crude product was used without further purification, or was precipitated with Et_2O and collected by suction.

General Procedure for Saponification of Methyl Esters (*GP 2*). The ester was placed in THF at 0°. After addition of an equal amount of 1M aq. LiOH (1.5 to 10 equiv.), the mixture was stirred at 0° for 1 h and at r.t. for another h. The pH was then adjusted to 7 with 1M aq. $KHSO_4$. The org. solvent was removed by evaporation at reduced pressure, and the resulting mixture was cooled to 0°, acidified to pH 2–3 with 1M aq. $KHSO_4$, and extracted with AcOEt (3×). The combined extracts were washed with brine and dried ($MgSO_4$). Evaporation of the solvent and drying *in vacuo* yielded the product.

General Procedure for Amide Bond Formation with HBTU (*GP 3*). HBTU (1 equiv.) was added to a stirred soln. of the carboxylic acid (1 equiv.) and EtN^iPr_2 (2 equiv.). After stirring for 30 min, the amine was added, and the reaction was allowed to proceed overnight under Ar. The mixture was diluted with the tenfold volume of AcOEt and successively washed with 1M aq. $KHSO_4$, sat. aq. $NaHCO_3$, and brine. Then, the org. layer was dried ($MgSO_4$) and concentrated under reduced pressure.

General Procedure for Amide Bond Formation with Brop (*GP 4*). The amine (1 equiv.), the carboxylic acid (1.12, 1.2, or 1.25 equiv.), and Et_3N (2.22 equiv.) were dissolved in CH_2Cl_2 or DMF at 0°. The reaction was started by the addition of Brop (1.12, 1.2, or 1.25 equiv.) and allowed to proceed overnight while stirring at r.t. under Ar.

H-Aeg(H)-OH (= N-*(2-Aminoethyl)glycine*; **1**). Ethylenediamine (823 g, 13.7 mol) was rapidly stirred while being cooled in an ice-bath. $ClCH_2COOH$ (131 g, 1.39 mol) was added portionwise during 8 h, thereby ensuring that each portion was dissolved before adding further material. The mixture was

stirred at r.t. for 16 h, concentrated *in vacuo*, and triturated with DMSO (2 l). After stirring overnight, an amorphous solid was isolated by suction, washed with DMSO and Et_2O, and dried *in vacuo:* **1** (116 g, 980 mmol, 70%). Colorless solid. ^{1}H-NMR (200 MHz, D_2O): 3.24 (*s*, CH_2 of Gly); 3.04–2.83 (*m*, CH_2CH_2). ^{13}C-NMR (50 MHz, D_2O): 178.26 (CO); 51.56 (CH_2 of Gly); 46.53 ($NH_2CH_2CH_2$); 38.56 (NH_2CH_2). FAB-MS: 119.1 (100, $[M+H]^+$).

(*2,4-Difluoro-5-methylphenyl)acetic Acid* (**2**). Mg Turnings (4.87 g, 200 mmol) were placed in dry Et_2O (10 ml). *1-(Bromomethyl)-2,4-difluoro-5-methylbenzene* (**4**; 1.11 g, 5.00 mmol) was added dropwise to start the reaction. A soln. of **4** (21.0 g, 95.0 mmol) in dry Et_2O (25 ml) was added dropwise over a period of 10 min, and the mixture was refluxed for 2.5 h. After cooling to $-10°$, a stream of dry CO_2 gas was directed through the mixture for 1 h, thereby keeping the temp. below 0°. The mixture was hydrolyzed with ice and 6M aq. HCl. The org. layer was separated, and the aq. phase was extracted with Et_2O. The combined org. phases were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by CC (SiO_2; $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 10:1) to give **2** (10.7 g, 57.5 mmol, 57%). Colorless solid. R_f (CH_2Cl_2) 0.20. ^{1}H-NMR (200 MHz, $CDCl_3$): 9.86 (br. *s*, COOH); 7.06 (*dd*, *t*-like, J = 8.3, 8.3, arom. H–C(6)); 6.78 (*dd*, *t*-like, J = 9.6, 9.6, arom. H–C(3)); 3.64 (*s*, CH_2); 2.22 (*s*, Me). ^{13}C-NMR (50 MHz, $CDCl_3$): 177.12 (CO); 160.56 (*dd*, ^1J(C,F) = 247, ^3J(C,F) = 11.5, arom. C(4)); 159.26 (*dd*, ^1J(C,F) = 247, ^3J(C,F) = 11.9, arom. C(2)); 133.37 (*dd*, ^3J(C,F) = 6.50, ^3J(C,F) = 5.40, arom. CH(6)); 121.10 (*dd*, ^2J(C,F) = 17.3, ^3J(C,F) = 3.80, arom. C(5)); 116.33 (*dd*, ^2J(C,F) = 15.7, ^4J(C,F) = 3.80, arom. C(1)); 103.54 (*dd*, *t*-like, ^2J(C,F) = 26.1, 26.1, arom. CH(3)); 33.77 (*d*, ^3J(C,F) = 2.70, CH_2); 14.02 (*d*, ^3J(C,F) = 3.50, Me). ^{19}F-NMR (565 MHz, (D_6)DMSO): −115.57 (*m*, F–C(4)); −117.17 (*m*, F–C(2)). EI-MS: 186.0 (32, $M^{+\bullet}$), 141.0 (100, $[M-COOH]^+$). HR-EI-MS: 186.0502 (30, $M^{+\bullet}$, $C_9H_8F_2O_2^+$; calc. 186.0492).

Compound **4**. Paraformaldehyde (4.93 g, 156 mmol) was dissolved in 33% HBr/AcOH soln. (80 ml) followed by the addition of *2,4-difluorotoluene* (**3**; 20.0 g, 156 mmol) and $ZnBr_2$ (15.8 g, 70.0 mmol). After stirring for 4 h at 120°, the mixture was cooled to r.t. and hydrolyzed with an equal volume of H_2O. The combined org. layers were successively washed with sat. aq. $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, cyclohexane) to give **4** (22.6 g, 120 mmol, 66%). Colorless oil. R_f (cyclohexane) 0.34. ^{1}H-NMR (200 MHz, $CDCl_3$): 7.20 (*dd*, *t*-like, J = 8.3, 8.3, arom. H–C(6)); 6.77 (*dd*, *t*-like, J = 9.6, 9.6, arom. H–C(3)); 4.46 (*s*, CH_2); 2.23 (*s*, Me). ^{13}C-NMR (50 MHz, $CDCl_3$): 161.28 (*dd*, ^1J(C,F) = 250, ^3J(C,F) = 11.9, arom. C(4)); 159.01 (*dd*, ^1J(C,F) = 250, ^3J(C,F) = 12.3, arom. C(2)); 133.21 (*dd*, ^3J(C,F) = 6.90, ^3J(C,F) = 4.20, arom. CH(6)); 120.91 (*dd*, ^2J(C,F) = 29.1, ^3J(C,F) = 4.20, arom. C(5)); 121.23 (*dd*, ^2J(C,F) = 26.1, ^4J(C,F) = 4.20, arom. C(1)); 103.89 (*dd*, ^2J(C,F) = 24.9, ^2J(C,F) = 26.5, arom. CH(3)); 25.39 (*d*, ^3J(C,F) = 3.80, CH_2); 14.01 (*d*, ^3J(C,F) = 3.10, Me). ^{19}F-NMR (565 MHz, (D_6)DMSO): −111.49 (*m*, F–C(4)); −115.88 (*m*, F–C(2)). EI-MS: 268 (72, $[M+2\,Na]^+$), 253 (100), 141.0 (78, $[M-Br]^+$).

Me_2-Aeg(Me)-OH (= N-*[2-(Dimethylamino)ethyl]*-N-*methylglycine*; **5a**). To a soln. of **1** (2.50 g, 21.2 mmol) in H_2O (200 ml) at 0°, a soln. of formalin (5.68 ml, 73.3 mmol) in H_2O (100 ml) was added dropwise. After stirring for 1 h at r.t., 10% Pd/C (2.00 g) was added, and the mixture was hydrogenated at 3.5 bar. Upon completion of the reaction, the mixture was filtered through a pad of diatomaceous earth and concentrated *in vacuo*. All volatiles were removed by repeated co-evaporation with toluene. The residue was crystallized from MeOH (20 ml) to give **5a** (2.54 g, 15.7 mmol, 75%). Colorless solid. For anal. data, see below.

Me_2-Aeg(Me)-OH · 2 HCl (**5a** · 2 HCl). To **1** (2.50 g, 21.2 mmol) at 0°, HCOOH (3.99 ml, 106 mmol), followed by formalin (5.68 ml, 73.3 mmol), was added. The resulting soln. was refluxed for 12 h, cooled to r.t., and treated with conc. aq. HCl (11.6 ml). The volatiles were evaporated *in vacuo*, and the residue was crystallized from MeOH (20 ml) to give **5a** · 2 HCl (2.02 g, 8.67 mmol, 41%). Colorless solid. ^{1}H-NMR (200 MHz, D_2O): 4.20 (*s*, CH_2 of Gly); 3.77 (*m*, CH_2CH_2); 3.11 (*s*, MeN); 3.04 (*s*, Me_2N). ^{13}C-NMR (50 MHz, D_2O): 168.26 (COOH); 57.31 (CH_2 of Gly); 51.29 (CH_2); 50.82 (CH_2); 43.57 (MeN); 42.17 (Me_2N). FAB-MS: 321.2 (10, $[2M+H]^+$), 161.1 (100, $[M+H]^+$).

Me_2-Aeg(Me)-Aeg(C)-Aeg(F)-Aeg(G)-OH · 3 TFA (= N-*[(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)acetyl]*-N-*[2-({*N-*[2-({*N-*[(4-amino-2-oxopyrimidin-1(2*H*)-yl)acetyl]*-N-*[2-({*N-*[2-(dimethylamino)ethyl]*-N-*methylglycyl}amino)ethyl]glycyl}amino)ethyl]*-N-*[(2,4-difluoro-5-methylphenyl)acetyl]glycyl}amino)ethyl]glycine*; **10**). *Me_2-Aeg(Me)-Aeg(C^Z)-Aeg(F)-Aeg(G^Z)-OBn · 2 TFA* (**49**; 305 mg,

214 mmol; see later), *m*-cresol (0.50 ml, 9.6 mmol), and thioanisole (0.50 ml, 8.5 mmol) were dissolved in TFA (3 ml). TFMSA (1 ml) was added dropwise, and the mixture was shaken for 2 h at r.t. The reaction was then quenched by the addition of Et_2O (45 ml), and the resulting suspension was cooled to 0°. The precipitate was sedimented by centrifugation and washed with Et_2O twice. Purification by RP-HPLC and subsequent freeze drying gave **10** (157 mg, 120 μmol, 56%). Colorless powder. ^{1}H-NMR (200 MHz, (D_6)DMSO, 140°): 7.63 (*d*, *J* = 7.4, H–C(6) of C); 7.57 (*s*, H–C(8) of G); under it (br. *s*, 3 amide NH); 7.16 (*t*, *J* = 8.7, 1 arom. H of F); 6.89 (*t*, *J* = 9.8, 1 arom. H of F); 6.21 (br. *s*, various R_3N^+H, COOH); 5.96 (*d*, *J* = 7.4, H–C(5) of C); 4.88 (*s*, CH_2); 4.65 (*s*, CH_2); 4.15 (*s*, CH_2); 4.04 (*s*, CH_2); 4.02 (*s*, CH_2); 3.65 (*s*, CH_2); 3.58–3.17 (*m*, 14 H of 4 CH_2CH_2, CH_2); 2.85 (*s*, Me_2N); under it (*m*, 2 H of 4 CH_2CH_2); 2.37 (*s*, Me); 2.17 (*s*, Me). ^{19}F-NMR (235 MHz, (D_6)DMSO, 30°; rotamers): −78.80 (*s*, TFA); −118.45 (*m*, F–C(4)); −120.99 (*m*, F–C(2)). MALDI-MS: 972.564 (485, $[M+2\,H]^+$). HR-ESI-MS: 973.4433 (13, $[M+3\,H]^+$, $C_{41}H_{59}F_2N_{16}O_{10}^+$; calc. 973.4568), 972.4411 (48, $[M+2\,H]^+$, $C_{41}H_{58}F_2N_{16}O_{10}^+$; calc. 972.4490), 971.4381 (100, $[M+H]^+$, $C_{41}H_{57}F_2N_{16}O_{10}^+$; calc. 971.4411).

H-Aeg(C)-Aeg(A)-Aeg(G)-Aem · 4 TFA (=*2-Amino*-N-*[12-(2-aminoethyl)-14-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-6-[2-(6-amino-9*H*-purin-9-yl)acetyl]-4,10,13-trioxo-3,6,9,12-tetraazatetradec-1-yl]-1,6-dihydro*-N-*(2-{[2-(morpholin-4-yl)ethyl]amino}-2-oxoethyl)-6-oxo-9*H*-purine-9-acetamide*; **11**). *Z-Aeg(C^Z)-Aeg(A^Z)-Aeg(G^Z)-Aem · TFA* (**36**; 491 mg, 0.308 mmol; see below), *m*-cresol (0.50 ml, 9.6 mmol), and thioanisole (0.50 ml, 8.5 mmol) were dissolved in TFA (3 ml). TFMSA (1 ml) was added dropwise, and the mixture was shaken for 2 h at r.t. The reaction was then quenched by the addition of Et_2O (45 ml), and the resulting suspension was cooled to 0°. The precipitate was sedimented by centrifugation and washed with Et_2O twice. Purification by RP-HPLC and subsequent freeze-drying gave **11** (238 mg, 170 μmol, 55%). Colorless powder. ^{1}H-NMR (200 MHz, (D_6)DMSO, 140°; rotamers): 8.56, 8.22, 8.21, 8.02 (4*s*, H–C(2) of A, H–C(8) of A); 7.88 (br. *m*, 3 amide NH); 7.76 (*m*, H–C(C(6) of C, H–C(8) of G); 6.42 (br. *s*, div. R_3N^+H); 6.88 (*m*, H–C(5) of C); 5.12 (br. *s*, base CH_2); 4.89 (*m*, base CH_2); 4.57 (br. *s*, base CH_2); 3.78–3.10 (*m*, 30 H, 2 NCH_2CH_2O, 4 CH_2CH_2, 3 CH_2 of Gly). MALDI-MS: 949.298 (169, $[M+2\,H]^+$). HR-ESI-MS: 950.4438 (13, $[M+3\,H]^+$, $C_{38}H_{56}N_{21}O_9^+$; calc. 950.4570), 949.4411 (51, $[M+2\,H]^+$, $C_{38}H_{55}N_{21}O_9^+$; calc. 949.4491), 948.4387 (100, $[M+H]^+$, $C_{38}H_{54}N_{21}O_9^+$; calc. 948.4413).

H-Aeg(H)-OMe · 2 HCl (=*Methyl* N-*(2-Aminoethyl)glycinate Dihydrochloride*; **13** · 2 HCl). To a suspension of **1** (93.0 g, 787 mmol) in MeOH (1.5 l) at 0°, $SOCl_2$ (138 ml, 1.90 mol) was added dropwise. After refluxing overnight, the volume was reduced to one-third, and Et_2O (500 ml) was added. The suspension was stirred for 30 min in an ice-bath, and the precipitate was collected by suction, washed with Et_2O, and dried *in vacuo* to give **13** · 2 HCl (130 g, 641 mmol, 81%). Colorless solid. ^{1}H-NMR (200 MHz, D_2O): 4.14 (*s*, CH_2 of Gly); 3.86 (*s*, MeO); 3.56–3.39 (*m*, CH_2CH_2). ^{13}C-NMR (50 MHz, D_2O): 167.73 (CO); 54.03 (MeO); 48.03 (CH_2 of Gly); 39.81 ($NH_2CH_2CH_2$); 35.79 (NH_2CH_2). FAB-MS: 154.0 (100, $[M+Na]^+$); 133.1 (70, $[M+H]^+$).

Z-Aeg(Boc)-OMe (=*Methyl* N-*(2-{[(Benzyloxy)carbonyl]amino}ethyl)*-N-*[(*tert-*butoxy)carbonyl]glycinate*; **14**). A soln. of Boc_2O (19.7 g, 93.8 mmol) in CH_2Cl_2 (90 ml) was added to a soln. of **17** (24.9 g, 93.8 mmol; see below) and Et_3N (13.1 ml, 93.8 mmol) in CH_2Cl_2 (410 ml) at 0°. After stirring overnight at r.t., the mixture was successively washed with 1M aq. $KHSO_4$, sat. aq. $NaHCO_3$, and brine (500 ml each), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give **14** (32.9 g, 89.9 mmol, 96%). Colorless oil. ^{1}H-NMR (200 MHz, (D_6)DMSO; 2 rotamers): 7.34 (*s*, 5 arom. H of Z); 7.18 (*m*, NH); 5.01 (*s*, CH_2 of Z); 3.93 (*s*, 0.90 H of CH_2 of Gly); 3.91 (*s*, 1.10 H of CH_2 of Gly); 3.65 (*s*, 1.30 H of MeO); 3.63 (*s*, 1.70 H of MeO); 3.07–3.29 (*m*, CH_2CH_2); 1.37 (*s*, 4.10 H of *t*-Bu); 1.32 (s, 4.90 H of *t*-Bu). ^{13}C-NMR (50 MHz, (D_6)DMSO; 2 rotamers): 170.61 (CO of Gly); 170.64 (CO of Gly); 156.07 (CO of Z); 154.89 (CO of Boc); 154.55 (CO of Boc); 137.21 (arom. C of Z); 137.10 (arom. C of Z); 128.34 (arom. *o*-CH of Z); 127.75 (arom. *m*-CH of Z); 127.66 (arom. *p*-CH of Z); 79.31 (Me_3C); 79.24 (Me_3C); 65.27 (CH_2 of Z); 65.23 (CH_2 of Z); 51.72 (MeO); 49.28, 48.38, 47.24, 47.14 (2 CH_2 of Gly, 2 $ZNHCH_2CH_2$); *the signal for ZNHCH$_2$ is expected at* ca. *40 ppm, and is probably hidden by the DMSO signal*; 27.88 (Me of Boc); 27.80 (Me of Boc). FAB-MS: 389.1 (26, $[M+Na]^+$), 367.1 (22, $[M+H]^+$), 267.0 (100, $[M-Boc]^+$).

Boc-Aeg(Alloc)-OMe (=*Methyl* N-*[(Allyloxy)carbonyl]*-N-*(2-{[(*tert-*butoxy)carbonyl]amino}-ethyl)glycinate*; **15**). *Boc-Aeg(H)-OMe* (**18**; 24.0 g, 103 mmol; see below) and Et_3N (14.4 ml, 103 mmol) were dissolved in CH_2Cl_2 (360 ml) at 0°. A soln. of Alloc-Cl (14.3 ml, 134 mmol) in CH_2Cl_2 (30 ml) was

then added dropwise. After stirring for 2 h at r.t., the solvent was evaporated *in vacuo*. The residue was taken up in AcOEt (500 ml) and successively washed with 1M aq. $KHSO_4$, sat. aq. $NaHCO_3$, and brine (500 ml each). The org. layer was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. Compound **15** (27.8 g, 87.9 mmol, 85%) was obtained as a colorless oil and used without further purification. ^{1}H-NMR (200 MHz, $CDCl_3$; 2 rotamers): 5.90 (*m*, CH_2CH=CH_2); 5.25 (*m*, NH, CH_2CH=CH_2); 4.60 (*m*, CH_2CH=CH); 4.00 (*s*, CH_2 of Gly); 3.75, 3.74 (2*s*, MeO); 3.46 (*m*, BocNHCH_2); 3.29 (*m*, BocNHCH$_2$CH_2); 1.43 (*s*, *t*-Bu). ^{13}C-NMR (50 MHz, $CDCl_3$; 2 rotamers): 170.83 (CO of Gly); 170.56 (CO of Gly); 156.36 (CO of Boc); 156.21 (CO of Alloc); 156.07 (CO of Alloc); 132.61 (CH_2CH=CH_2); 117.88 (CH_2CH=CH_2); 117.42 (CH_2CH=CH_2); 79.34 (Me_3C); 66.66 (CH_2CH=CH_2); 66.43 (CH_2CH=CH_2); 52.36 (MeO); 49.94 (CH_2 of Gly); 49.69 (CH_2 of Gly); 49.02 (BocNHCH$_2$CH$_2$); 48.69 (BocNHCH$_2$CH$_2$); 39.19 (BocNHCH_2); 28.46 (Me of Boc). FAB-MS: 655.4 (5, $[2M+Na]^+$), 633.4 (3, $[2M+H]^+$), 339.2 (25, $[M+Na]^+$), 317.2 (23, $[M+H]^+$), 217.1 (100, $[M-Boc]^+$).

Boc-Aeg(Fmoc)-OMe (= *Methyl* N-*(2-{[(*tert-*Butoxy)carbonyl]amino}ethyl)*-N-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}glycinate*; **16**). Compound **18** (26.5 g, 114 mmol) and EtN^iPr_2 (39.7 ml, 228 mmol) were dissolved in CH_2Cl_2 (400 ml). After cooling to 0°, a soln. of Fmoc-Cl (29.4 g, 144 mmol) in CH_2Cl_2 (100 ml) was added. The mixture was stirred for 1 h at 0° and for additional 3 h at r.t. After washing with 1M aq. $KHSO_4$, sat. aq. $NaHCO_3$, and brine, the org. phase was dried ($MgSO_4$). Evaporation of the solvent, followed by CC (SiO_2; CH_2Cl_2/MeOH 50:1) gave **16** (44.0 g, 96.9 mmol, 86%). Colorless oil. R_f (CH_2Cl_2/MeOH 50:1) 0.21. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°): 7.85 (*m*, 2 arom. H of Fmoc); 7.62 (*m*, 2 arom. H of Fmoc); 7.46–7.29 (*m*, 4 arom. H of Fmoc); 6.21 (br. *m*, NH); 4.39 (*m*, CH_2 of Fmoc); 4.26 (*m*, CH of Fmoc); 3.97 (*s*, CH_2 of Gly), 3.65 (*s*, Me), 3.33 (*m*, 2 H of CH_2CH_2), 3.09 (*m*, 2 H of CH_2CH_2); 1.39 (*s*, *t*-Bu). ^{13}C-NMR (50 MHz, (D_6)DMSO, 110°): 169.25 (*C*OOMe); 154.95 (CO of Fmoc, CO of Boc); 143.32 (arom. C of Fmoc); 140.27 (arom. C of Fmoc); 126.94 (arom. CH of Fmoc); 126.40 (arom. CH of Fmoc); 124.20 (arom. CH of Fmoc); 119.30 (arom. CH of Fmoc); 77.30 (Me_3C), 66.56 (CH_2 of Fmoc); 50.96 (MeO); 48.47, 47.31, 46.41, 38.26 (CH of Fmoc, CH_2 of Gly, CH_2CH_2); 27.65 (Me of Boc). FAB-MS: 477 (15, $[M+Na]^+$), 455 (5, $[M+H]^+$), 355.1 (67, $[M-Boc]^+$), 178.0 (100).

Z-Aeg(H)-OMe (= *Methyl* N-*(2-{[(Benzyloxy)carbonyl]amino}ethyl)glycinate*; **17**). To a soln. of Z-OSu (37.1 g, 150 mmol) in MeCN (600 ml) at −15° **13**·2 HCl (30.4 g, 150 mmol), followed by NMM (41.1 ml, 373 mmol), was added. The mixture was stirred at −15° for 3 h, followed at r.t. overnight. After evaporation of volatiles *in vacuo*, the residue was taken up in 1M aq. $KHSO_4$ (600 ml) and washed with AcOEt (600 ml). The pH was adjusted to 8–9 with solid $NaHCO_3$, and the aq. soln. was washed with AcOEt (3 × 500 ml). The combined org. phases were washed with brine, dried ($MgSO_4$), filtered, and concentrated to give **17** (25.0 g, 93.9 mmol, 63%). Colorless oil. ^{1}H-NMR (200 MHz, $CDCl_3$; 2 rotamers): 7.38–7.13 (*m*, 5 arom. H of Z); 5.32 (br. *s*, NH of Z); 5.02 (*s*, CH_2 of Z); 3.64 (*s*, MeO); 3.32 (*s*, CH_2 of Gly); 3.20 (*dt*, *J* = 5.7, 5.7, ZNHCH_2); 2.68 (*t*, *J* = 5.7, ZNHCH$_2$CH_2). ^{13}C-NMR (50 MHz, $CDCl_3$; 2 rotamers): 172.95 (CO of Gly); 156.55 (CO of Z); 136.63 (arom. C of Z); 128.51 (arom. *o*-CH of Z); *ca.* 128.07 (2*s* (not resolved), arom. *p*-CH of Z, arom. *m*-CH of Z); 66.65 (CH_2 of Z); 51.86 (MeO); 50.23 (CH_2 of Gly); 48.63 (CH_2 of Z); 40.63 (ZNH*C*H$_2$). FAB-MS: 289.0 (11, $[M+Na]^+$), 267.1 (100, $[M+H]^+$).

Boc-Aeg(H)-OMe (= *Methyl* N-*(2-{[(*tert-*Butoxy)carbonyl]amino}ethyl)glycinate*; **18**). Compound **13**·2 HCl (20.0 g, 98.5 mmol) was suspended in CH_2Cl_2 (1.2 l) at −10°, followed by the addition of Et_3N (27.3 ml, 197 mmol). After adding a soln. of Boc_2O (21.5 g, 98.5 mmol) in CH_2Cl_2 (300 ml) dropwise during 30 min, the mixture was stirred at r.t. overnight. The mixture was successively washed with H_2O and brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was distilled under reduced pressure to give **18** (14.4 g, 62.0 mmol, 63%). Colorless oil. R_f (CH_2Cl_2/MeOH 10:1) 0.55. R_f (AcOEt/MeOH 4:1) 0.45. ^{1}H-NMR (200 MHz, $CDCl_3$): 3.72 (*s*, MeO); 3.40 (*s*, CH_2 of Gly); 3.20 (*dt*, *q*-like, *J* = 5.8, 5.8, BocNHCH_2); 2.73 (*t*, *J* = 5.8, BocNHCH$_2$CH_2); 1.43 (*s*, *t*-Bu). ^{13}C-NMR (50 MHz, $CDCl_3$): 173.04 (CO of Gly); 156.21 (CO of Boc); 79.34 (Me_3C); 51.97 (MeO); 50.41 (CH_2 of Gly); 48.90, 41.02 (CH_2CH_2); 28.53 (Me of Boc). FAB-MS: 255.1 (19, $[M+Na]^+$), 233.1 (100, $[M+H]^+$).

Boc-Aeg(Alloc)-OH (= N-*[(Allyloxy)carbonyl-]*-N-*(2-{[(*tert-*butoxy)carbonyl]amino}ethyl)glycine*; **19**). Compound **15** (27.8 g, 87.8 mmol) was dissolved in THF (180 ml) and treated with an equal volume of 1M aq. LiOH according to *GP 1*. After workup, the product **15** (25.1 g, 83.0 mmol, 94%) had sufficient purity to be used in the next synthetic step. ^{1}H-NMR (200 MHz, (D_6)DMSO; 2 rotamers):

12.67 (br. *s*, COOH); 6.75 (br. *m*, NH); 6.01–5.73 (*m*, CH_2CH=CH_2); 5.34–5.11 (*m*, CH_2CH=CH_2); 4.51 (*m*, CH_2CH=CH); 3.92, 3.89 (2*s*, CH_2 of Gly); 3.33–3.05 (*m*, CH_2CH_2); 1.36 (*s*, *t*-Bu). ^{13}C-NMR (50 MHz, (D_6)DMSO; 2 rotamers): 171.18 (COOH); 171.07 (COOH); 155.59, 155.46, 155.20 (CO of Boc, CO of Alloc); 133.22 (CH_2CH=CH_2); 116.70 (CH_2CH=CH_2); 116.34 (CH_2CH=CH_2); 77.67 (Me_3C); 65.34 (CH_2CH=CH_2); 65.13 (CH_2CH=CH_2); 48.97 (CH_2 of Gly); 48.81 (CH_2 of Gly); 47.78 ($BocNHCH_2CH_2$); 47.13 ($BocNHCH_2CH_2$); 38.36 ($BocNHCH_2$); 38.06 ($BocNHCH_2$); 28.18 (Me of Boc). FAB-MS: 325.1 (100, $[M+Na]^+$), 303 (19, $[M+H]^+$), 203.1 (73, $[M-Boc]^+$).

H-Aeg(Fmoc)-OMe · TFA (= *Methyl* N-*(2-Aminoethyl)*-N-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-glycinate*; **20**). Compound **16** (44.0 g, 96.6 mmol) was treated with CH_2Cl_2 (100 ml) and TFA (100 ml) according to in *GP 1*. After precipitation with Et_2O, an amorphous solid was isolated by suction. Crude **20** (41.2 g, 87.8 mmol, 91%) was directly used in the next step. FAB-MS: 709.1 (5, $[2M+H]^+$), 377.0 (11, $[M+Na]^+$), 355.1 (100, $[M+H]^+$).

Boc-Aeg(Alloc)-Aeg(Fmoc)-OMe (= *Methyl* N-*(2-{[*N-*(2-{[(*tert-*Butoxy)carbonyl]amino}ethyl)*-N-*[(prop-2-en-1-yloxy)carbonyl]glycyl]amino}ethyl)*-N-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}glycinate*; **21**). Compounds **19** (13.9 g, 45.8 mmol) and **20** (21.5 g, 45.8 mmol) were treated according to *GP 3*. The crude product was purified by CC (SiO_2; AcOEt/MeOH 9:1) to give **21** (22.4 g, 35.0 mmol, 76%). Colorless foam. R_f (AcOEt/MeOH 9:1) 0.6. ^{1}H-NMR (250 MHz, (D_6)DMSO, 100°; 2 rotamers, the Fmoc group is not in the regime of fast exchange): 7.87 (*m*, 2 arom. H of Fmoc); 7.61 (*d*, $J=7.4$, 2 arom. H of Fmoc); 7.5 (br. *m*, NH); 7.45–7.29 (*m*, 4 arom. H of Fmoc); 6.35 (br. *t*, NH); 5.90 (*ddt*, $J=17$, 11, 5.2, CH_2CH=CH_2); 5.27 (*ddt*, *dq*-like, $J=17$, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.15 (*ddt*, *dq*-like, $J=11$, 1.6, 1.6, CH_2CH=CH_ZH_E); 4.51 (*dt*, $J=5.2$, 1.6, 2 H, CH_2CH=CH_2); 4.39 (*m*, CH_2 of Fmoc); 4.26 (*m*, CH of Fmoc); 3.97 (*s*, N(Alloc)CH_2CONH); 3.82 (*s*, 1.6 H of N(Fmoc)CH_2COOMe); 3.74 (*s*, 0.4 H of N(Fmoc)CH_2COOMe); 3.65, 3.64 (2*s*, MeO); 3.36–3.08 (*m*, 2 CH_2CH_2); 1.39 (*s*, *t*-Bu). ^{13}C-NMR (50 MHz, (D_6)DMSO; 2 rotamers, the Fmoc group is not in the regime of fast exchange): 169.39 (COMe); 168.32 (CO of Gly); 155.03, 155.01 (2 CO); 143.34 (arom. C of Fmoc); 140.31 (arom. C of Fmoc); 132.79 (CH_2CH=CH_2); 127.01 (arom. CH of Fmoc); 126.47 (arom. CH of Fmoc); 124.26 (arom. CH of Fmoc); 119.38 (arom. CH of Fmoc); 116.08 (CH_2CH=CH_2); 77.24 (Me_3C); 66.64 (CH_2CH=CH_2); 64.79 (CH_2 of Fmoc); 51.07 (Me); 50.02 (CH_2); 49.32 (CH_2); 48.58 (CH_2); 48.62 (CH_2); 47.65 (CH_2); 47.08 (CH_2); 46.40 (CH of Fmoc); 38.18 (CH_2); 36.90 (CH_2); 36.91 (CH_2); 27.71 (Me of Boc). FAB-MS: 678.3 (47, $[M+K]^+$), 661.3 (97, $[M+Na]^+$), 539.3 (33, $[M-Boc]^+$), 178.1 (100).

Z-Aeg(Boc)-Aeg(Alloc)-Aeg(Fmoc)-OMe (= *Methyl* N-*[2-({[({*N-*[(Allyloxy)carbonyl]*-N-*(2-{[*N-*(2-{[(benzyloxy)carbonyl]amino}ethyl)*-N-*{[((*tert-*butoxy)carbonyl]glycyl}amino)ethyl]glycyl}amino)ethyl]*-N-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}glycinate*; **22**). *Z-Aeg(Boc)-OH* (**24**; 12.3 g, 34.9 mmol; see below) and *H-Aeg(Alloc)-Aeg(Fmoc)-OMe* (**23**; 22.8 g, 34.9 mmol; see below) were coupled according to *GP 3*. Purification by CC (SiO_2; AcOEt/MeOH 9:1) afforded **22** (19.0 g, 21.7 mmol, 62%). Colorless foam. R_f (AcOEt/MeOH 9:1) 0.28. ^{1}H-NMR (250 MHz, (D_6)DMSO, 100°): 7.85 (*d*, $J=7.0$, 2 arom. H of Fmoc); 7.61 (*d*, $J=7.5$, 2 arom. H of Fmoc); under it (br. *s*, NH); 7.57 (br. *s*, NH); 7.44–7.28 (*m*, 4 arom. H of Fmoc, 5 arom. H of Z); 6.81 (br. *t*, NH); 5.90 (*ddt*, $J=17$, 11, 5.2, CH_2CH=CH_2); 5.28 (*ddt*, *dq*-like, $J=17$, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.15 (*ddt*, *dq*-like, $J=11$, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.04 (*s*, CH_2 of Z); 4.52 (*dt*, $J=5.2$, 1.6, CH_2CH=CH_2); 4.39 (*m*, CH_2 of Fmoc); 4.26 (br. *t*, $J=6.2$, CH of Fmoc); 3.97 (*s*, CH_2 of Gly); 3.84 (*s*, CH_2 of Gly); 3.74 (*s*, CH_2 of Gly); 3.64 (*s*, Me); 3.35–3.15 (*m*, 3 CH_2CH_2); 1.38 (*s*, *t*-Bu). ^{13}C-NMR (63 MHz, (D_6)DMSO, 100°): 169.37 (COOMe); 168.65 (CO of Gly); 168.38 (CO of Gly); 155.51, 155.04, 155.00, 154.48 (CO of Z, CO of Boc, CO of Alloc, CO of Fmoc); 143.34 (arom. C of Fmoc); 140.32 (arom. C of Fmoc); 136.78 (arom. C of Z); 132.75 (CH_2CH=CH_2); 127.66 (arom. CH of Fmoc); 127.01 (arom. *o*-CH of Z); 126.90 (arom. *m*-CH of Z); 126.47 (arom. *p*-CH of Z); 124.26 (arom. CH of Fmoc); 119.38 (arom. CH of Fmoc); 116.16 (CH_2CH=CH_2); 78.59 (Me_3C); 66.63 (CH_2CH=CH_2); 64.84 (CH_2 of Fmoc); 51.07 (Me); 46.40 (CH of Fmoc); *signals of the remaining CH_2 groups are located in the range of 51.13–37.74 ppm, but cannot be separated unambiguously from the baseline under the given conditions* (76 mM sample, 1024 scans); 27.50 (Me of Boc). FAB-MS: 895.3 (35, $[M+Na]^+$), 873.3 (15, $[M+H]^+$), 795.3 (3, $[M-Boc+Na]^+$), 773.3 (36, $[M-Boc]^+$), 91.0 (100).

H-Aeg(Alloc)-Aeg(Fmoc)-OMe · TFA (= *Methyl* N-*[2-({*N-*[(Allyloxy)carbonyl]*-N-*(2-aminoethyl)-glycyl}amino)ethyl]*-N-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}glycinate*; **23**). Compound **21** (22.4 g,

35.0 mmol) was treated with CH_2Cl_2 (40 ml) and TFA (40 ml) according to *GP 1*. The resulting oil (22.8 g, 34.9 mmol, 99%) was directly used in the next step. FAB-MS: 561.2 (17, $[M+Na]^+$), 539.2 (100, $[M+H]^+$).

Z-Aeg(Boc)-OH (= N-*(2-{[(Benzyloxy)carbonyl]amino}ethyl)*-N-*[(*tert-*butoxy)carbonyl]glycine*; **24**). The product was prepared from a soln. of **14** (32.9 g, 89.9 mmol) in THF (180 ml) according to *GP 2*. After adjusting the pH to 2–3, the product precipitated as an amorphous solid. The precipitate was collected by suction, washed with H_2O and Et_2O, and dried *in vacuo* to give **24** (29.4 g, 83.4 mmol, 93%). Colorless solid. ^{1}H-NMR (200 MHz, (D_6)DMSO; 2 rotamers): 12.61 (br. *s*, COOH); 7.34 (*m*, 5 arom. H of Z); 7.17 (br. *m*, NH of Z); 5.01 (*s*, CH_2 of Z); 3.86 (*s*, 1 H of CH_2 of Gly); 3.82 (*s*, 1 H of CH_2 of Gly); 3.35–3.11 (*m*, CH_2CH_2); 1.37 (*s*, 4 H of *t*-Bu); 1.34 (*s*, 5 H of *t*-Bu). ^{13}C-NMR (50 MHz, (D_6)DMSO; 2 rotamers): 171.61 (CO of Gly); 171.46 (CO of Gly); 156.16 (CO of Z); 156.09 (CO of Z); 154.93 (CO of Boc); 154.78 (CO of Boc); 137.24 (arom. C of Z); 137.13 (arom. C of Z); 128.36 (arom. *o*-CH of Z); 127.75 (arom. *m*-CH of Z); 127.65 (arom. *p*-CH of Z); 79.09 (Me_3C); 79.07; (Me_3C); 65.28 (CH_2 of Z); 65.22 (CH_2 of Z); 49.30 (CH_2 of Gly); 48.44 (CH_2 of Gly); 47.26 ($ZNHCH_2CH_2$); 47.16 ($ZNHCH_2CH_2$); *the signal for ZNHCH$_2$ is expected at* ca. *40 ppm, and is probably hidden by the DMSO signal*; 27.93 (Me of Boc), 27.87 (Me of Boc). FAB-MS: 375.1 (15, $[M+Na]^+$), 353.1 (24, $[M+H]^+$), 297.1 (25, $[M-t\text{Bu}]^+$), 253.1 (81, $[M-\text{Boc}]^+$), 91.0 (100).

Z-Aeg(Boc)-Aeg(Alloc)-Aeg(Fmoc)-OH (= N-*[2-({[{*N-*[(Allyloxy)carbonyl]*-N-*(2-{[*N-*(2-{[(benzyloxy)carbonyl]amino}ethyl)*-N-*{[(*tert-*butoxy)carbonyl]glycyl}amino)ethyl]glycyl}amino)ethyl]*-N-*{[(9*H-*fluoren-9-yl)methoxy]carbonyl}glycine*; **25**). Compound **22** (19.0 g, 21.7 mmol) was dissolved in THF (43.4 ml) at 0°; 1M aq. LiOH (43.4 ml) was added, and the mixture was stirred at 0° for 1 h and an additional h at r.t. Fmoc Elimination, which had taken place to some extent, was reversed by addition of Fmoc-Cl (7.31 g, 28.2 mmol, 1.3 equiv.) and stirring for 1 h at 0°. The mixture was then adjusted to pH 3 with a 1M aq. $KHSO_4$ and extracted with AcOEt (3 × 150 ml). The combined org. phases were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by CC (SiO_2, AcOEt/MeOH 9:1 → 1:1) to give **25** (12.9 g, 15.0 mmol, 69%). Colorless foam. R_f (AcOEt/MeOH 1:1) 0.46. ^{1}H-NMR (250 MHz, (D_6)DMSO; rotamers): 12.74 (br. *s*, COOH); 8.00 (br. *s*, 2 NH); 7.89 (br. *d*, *J* = 7.4, 2 arom. H of Fmoc); 7.67 (*d*, *J* = 7.3, 1 arom. H of Fmoc); 7.62 (*d*, *J* = 7.5, 1 arom. H of Fmoc); 7.45–7.23 (*m*, 4 arom. H of Fmoc, 5 arom. H of Z, NH); 6.01–5.76 (*m*, CH_2CH=CH_2); 5.20 (*m*, CH_2CH=CH_2); 5.00 (*s*, CH_2 of Z); 4.50 (*m*, CH_2CH=CH_2); 4.27 (*m*, CH_2 of Fmoc, CH of Fmoc); 4.04–3.68 (*m*, 3 CH_2 of Gly); 3.39–3.13 (*m*, 3 CH_2CH_2); 1.36, 1.31 (2*s*, Me_3C). *Elevated temps. led to fast Fmoc elimination, probably due to formation of a cyclic anhydride with the carboxy. Therefore, the characterization was not applicable in the overall fast regime.* FAB-MS: 881.2 (45, $[M+Na]^+$), 859.2 (13, $[M+H]^+$), 759.2 (28, $[M-\text{Boc}]^+$), 91.0 (100).

Z-Aeg(Boc)-Aeg(Alloc)-Aeg(Fmoc)-Aem (= N-*[15-[(Allyloxy)carbonyl]-9-[(*tert-*butoxy)carbonyl]-3-{[(9*H-*fluoren-9-yl)methoxy]carbonyl}-20-(morpholin-4-yl)-5,11,17-trioxo-3,6,9,12,15,18-hexaazaicos-1-yl]carbamic Acid Phenylmethyl Ester*; **27**). Compound **25** (12.8 g, 14.9 mmol) and HOSu (2.56 g, 22.3 mmol, 1.5 equiv.) were dissolved in CH_2Cl_2 (60 ml) at 0° under Ar, followed by the addition of DCC (3.38 g, 16.4 mmol, 1.1 equiv.). The mixture was allowed to warm to r.t. while stirring overnight. After cooling to −15°, *4-(2-aminoethyl)morpholine* (**26**; 1.96 ml, 14.9 mmol, 1 equiv.) was added, and stirring was continued for 1 h at −15° and additional 3 h at r.t. The dicyclohexylurea was filtered off, washed with CH_2Cl_2, and the filtrate was concentrated under reduced pressure. The residue was taken up in AcOEt (500 ml), successively washed with sat. aq. $NaHCO_3$ and brine (252 ml each), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by CC (SiO_2; AcOEt/MeOH 2:1) to give **27** (12.1 g, 12.5 mmol, 84%). Colorless foam. R_f (AcOEt/MeOH 2:1) 0.32. ^{1}H-NMR (250 MHz, (D_6)DMSO, 100°): 7.85 (*d*, *J* = 7.4, 2 arom. H of Fmoc); 7.72 (br. *t*, NH); 7.64 (*d*, *J* = 7.3, 2 arom. H of Fmoc); under it (NH); 7.48–7.26 (*m*, 4 arom. H of Fmoc, 5 arom. H of Z, NH); 6.81 (br. *t*, NH); 5.90 (*ddt*, *J* = 17, 11, 5.2, CH_2CH=CH_2); 5.27 (*ddt*, *dq*-like, *J* = 17, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.15 (*ddt*, *dq*-like, *J* = 11, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.04 (*s*, CH_2 of Z); 4.52 (br. *dt*, CH_2CH=CH_2); 4.30 (*m*, CH_2 of Fmoc, CH of Fmoc); 3.88 (*s*, CH_2 of Gly); 3.85 (*s*, CH_2 of Gly); 3.74 (*s*, CH_2 of Gly); 3.54 (*m*, 2 NCH_2CH_2O); 3.37–3.11 (*m*, 3.5 CH_2CH_2); 2.38 (*m*, 2 NCH_2CH_2O, 0.5 CH_2CH_2); 1.38 (*s*, *t*-Bu). *Elevated temps. led to efficient Fmoc elimination, probably due to the presence of the tertiary amine. Therefore, the characterization by ^{13}C-NMR spectroscopy was not possible.* MALDI-MS: 971.917 (68, $[M+H]^+$), 871.771 (248,

$[M-\text{Boc}]^+$). HR-ESI-MS: 973.4910 (17, $[M+3\,H]^+$, $C_{50}H_{69}N_8O_{12}^+$; calc. 973.5034), 972.4885 (58, $[M+2\,H]^+$, $C_{50}H_{68}N_8O_{12}^+$; calc. 972.4956), 971.4853 (100, $[M+H]^+$, $C_{50}H_{67}N_8O_{12}^+$; calc. 971.4878).

Z-Aeg(Boc)-Aeg(Alloc)-Aeg(H)-Aem (= N-*[9-[(Allyloxy)carbonyl]-3-[(*tert-*butoxy)carbonyl]-20-(morpholin-4-yl)-5,11,17-trioxo-3,6,9,12,15,18-hexaazaeicos-1-yl]carbamic Acid Phenylmethyl Ester*; **31**). Compound **27** (11.1 g, 11.4 mmol) was dissolved in CH_2Cl_2 at r.t. and treated with Et_2NH (17.8 ml, 171 mmol). After stirring for 1.5 h, the solvent was evaporated *in vacuo*, and the residue was purified by CC (SiO_2; AcOEt/MeOH 1:1 → 0:1) to give **31** (6.78 g, 9.05 mmol, 79%). Colorless foam. R_f (AcOEt/MeOH 1:1) 0.15. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°): 7.73 (br. *t*, NH); 7.66 (br. *t*, NH); 7.54 (br. *m*, NH); 7.34 (*m*, 5 arom. H of Z); 6.88 (br. *t*, NH); 5.89 (*ddt*, *J* = 17, 11, 5.2, CH_2CH=CH_ZH_E); 5.28 (*ddt*, *dq*-like, *J* = 17, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.16 (*ddt*, *dq*-like, *J* = 11, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.04 (*s*, CH_2 of Z); 4.52 (*dt*, *J* = 5.2, 1.6, CH_2CH=CH_2); 3.86 (*s*, CH_2 of Gly); 3.73 (*s*, CH_2 of Gly); 3.74 (*s*, CH_2 of Gly); 3.57 (*m*, 2 NCH_2CH_2O); 3.38–3.14 (*m*, 3.5 CH_2CH_2); 3.10 (*s*, CH_2 of Gly); 2.40 (*m*, 2 NCH_2CH_2O, 0.5 CH_2CH_2); 1.38 (*s*, *t*-Bu). FAB-MS: 771.3 (21, $[M+\text{Na}]^+$), 749.4 (59, $[M+H]^+$), 649.3 (13, $[M-\text{Boc}]^+$), 91 (100). HR-ESI-MS: 751.4239 (10, $[M+3\,H]^+$, $C_{35}H_{59}N_8O_{10}^+$; calc. 751.4354), 750.4212 (40, $[M+2\,H]^+$, $C_{35}H_{58}N_8O_{10}^+$; calc. 750.4276), 749.4178 (100, $[M+H]^+$, $C_{35}H_{57}N_8O_{10}^+$; calc. 749.4197).

Z-Aeg(Boc)-Aeg(Alloc)-Aeg(G^Z)-Aem (= *9-[(Allyloxy)carbonyl]-*N*-{15-[2-(1,6-dihydro-6-oxo-2-{[(phenylmethoxy)carbonyl]amino}-9*H*-purin-9-yl)acetyl]-3-[(*tert-*butoxy)carbonyl]-20-(morpholin-4-yl)-5,11,17-trioxo-3,6,9,12,15,18-hexaazaicos-1-yl}carbamic Acid Phenylmethyl Ester*; **32**). Compound **31** (6.78 g, 9.05 mmol) and *G^Z-AcOH* (**28**; 3.88 g, 11.3 mmol) were coupled in CH_2Cl_2 (35 ml) according to *GP 4*. The mixture was diluted with $CHCl_3$ and successively washed with sat. aq. $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by CC (SiO_2; AcOEt/MeOH 1:1 → 0:1) to give **32** (8.15 g, 7.78 mmol, 86%). Colorless foam. R_f (AcOEt/MeOH 1:1) 0.10. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°): 7.78 (*s*, H–C(8) of G); 7.73 (br. *t*, NH); 7.66 (br. *t*, NH); 7.54 (br. *m*, NH); 7.40–7.27 (*m*, 10 arom. H of Z); 6.77 (br. *t*, NH); 5.89 (*ddt*, *J* = 17, 11, 5.2, CH_2CH=CH_ZH_E); 5.28 (*s*, CH_2 of Z of G); 5.27 (*ddt*, *dq*-like, *J* = 17, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.15 (*ddt*, *dq*-like, *J* = 11, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.04 (*s*, CH_2 of Z); 4.98 (br. *s*, CH_2–N(9) of G); 4.51 (*dt*, *J* = 5.2, 1.6, CH_2CH=CH_2); 4.06, 3.95 (2 br. *s*, CH_2 of Gly); 3.87 (br. *s*, CH_2 of Gly); 3.74 (*s*, CH_2 of Gly); 3.55 (*m*, 2 NCH_2CH_2O); 3.35–3.14 (*m*, 3.5 CH_2CH_2); 2.41 (*m*, 2 NCH_2CH_2O, 0.5 CH_2CH_2); 1.38 (*s*, *t*-Bu). FAB-MS: 1096 (42, $[M+\text{Na}]^+$), 1074.6 (31, $[M+H]^+$), 154.0 (100). HR-ESI-MS: 1077.5062 (5, $[M+4\,H]^+$, $C_{50}H_{71}N_{13}O_{14}^+$; calc. 1077.5243), 1076.5035 (20, $[M+3\,H]^+$, $C_{50}H_{70}N_{13}O_{14}^+$; calc. 1076.5165), 1075.5010 (57, $[M+2\,H]^+$, $C_{50}H_{69}N_{13}O_{14}^+$; calc. 1075.5087), 1074.4983 (100, $[M+H]^+$, $C_{50}H_{68}N_{13}O_{14}^+$; calc. 1074.5008).

Z-Aeg(Boc)-Aeg(H)-Aeg(G^Z)-Aem (= N-*{6,9-Dihydro-9-[15-[(*tert-*butoxy)carbonyl]-3-(2-{[2-(morpholin-4-yl)ethyl]amino}-2-oxoethyl)-2,7,13,19-tetraoxo-21-phenyl-20-oxa-3,6,9,12,15,18-hexaazaheneicos-1-yl]-6-oxo-1*H*-purin-2-yl}carbamic Acid Phenylmethyl Ester*; **33**). Compound **32** (8.98 g, 8.58 mmol) and Et_2NH (26.7 ml, 257 mmol, 30 equiv.) were dissolved in CH_2Cl_2 (60 ml) at 0°. After the addition of $Pd[P(Ph)_3]_4$ (991 mg, 0.858 mmol), the mixture was stirred at r.t. for 1 h. The volatiles were evaporated *in vacuo*, and the residue was purified by CC (SiO_2; AcOEt/MeOH 1:1 → 0:1) to give **33** (5.21 g, 5.27 mmol, 61%). Colorless foam. R_f (MeOH) 0.19. ^{1}H-NMR (400 MHz, (D_6)DMSO; rotamers): 8.17 (br. *s*, NH); 7.86 (br. *s*, 2 NH); 7.79, 7.78 (2*s*, H–C(8) of G); 7.42–7.28 (*m*, 10 arom. H of Z); 7.24 (br. *t*, NH); 5.233, 5.226 (2*s*, CH_2 of Z of G); 5.00 (*s*, PhCH_2); 5.05, 4.93 (2*s*, CH_2–N(9) of G); 4.15, 3.92 (2*s*, CH_2 of Gly); 3.75, 3.70 (2*s*, CH_2 of Gly); 3.56–3.06 (*m*, CH_2 of Gly, 3.5 CH_2CH_2, 2 NCH_2CH_2O); 2.35 (*m*, 2 NCH_2CH_2O, 0.5 CH_2CH_2); 1.36, 1.31 (2*s*, Me_3C). *Elevated temps. led to line broadening that complicated the analysis.* MALDI-MS: 991.208 (120, $[M+H]^+$), 1013.249 (210, $[M+\text{Na}]^+$).

Z-Aeg(Boc)-Aeg(A^Z)-Aeg(G^Z)-Aem (= N-*(6,9-Dihydro-9-{15-[(*tert-*butoxy)carbonyl]-3-(2-{[2-(morpholin-1-yl)ethyl]amino}-2-oxoethyl)-2,7,13,19-tetraoxo-21-phenyl-9-[2-(6-{[(phenylmethoxy)carbonyl]amino}-9*H*-purin-9-yl)acetyl]-20-oxa-3,6,9,12,15,18-hexaazaheneicos-1-yl}-6-oxo-1*H*-purin-2-yl)-carbamic Acid Phenylmethyl Ester*; **34**). Compound **33** (5.21 g, 5.26 mmol) and *A^Z-AcOH* (**29**; 2.15 g, 6.58 mmol) were coupled in DMF according to *GP 4*. The mixture was concentrated *in vacuo* with a dry-ice evaporator, and the residue was purified by CC (SiO_2; MeOH) to give **34** (4.62 g, 3.56 mmol, 67%). Colorless foam. R_f (MeOH) 0.14. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°; rotamers): 8.57, 8.23, 8.14, 7.96 (4*m*, H–C(2) of A, H–C(8) of A); 7.92–7.17 (*m*, 15 arom. H of Z, H–C(8) of G, NH); 6.79 (br. *t*, NH); 6.66 (br. *s*, NH); 6.09 (br. *s*, NH); 5.25 (*s*, CH_2 of Z); 5.16 (br. *s*, CH_2–N(9)); 5.03 (*s*, CH_2 of Z); 4.87 (br. *s*,

CH_2–N(9)); 4.52 (*s*, CH_2 of Z); 4.23 (br. *s*, 2 CH_2 of Gly); 3.81, 3.76 (2 br. *s*, CH_2 of Gly); 3.57–3.18 (*m*, 2 NCH_2CH_2O, 3.5 CH_2CH_2); 2.41 (*m*, 2 NCH_2CH_2O, 0.5 CH_2CH_2); 1.38 (*s*, *t*-Bu). MALDI-MS: 1322.861 (127, $[M+Na]^+$), 1300.814 (171, $[M+2\,H]^+$). HR-ESI-MS: 1302.5703 (8, $[M+4\,H]^+$, $C_{61}H_{78}N_{18}O_{15}^+$; calc. 1302.5894), 1301.5671 (26, $[M+3\,H]^+$, $C_{61}H_{77}N_{18}O_{15}^+$; calc. 1301.5816), 1300.5652 (75, $[M+2\,H]^+$, $C_{61}H_{76}N_{18}O_{15}^+$; calc. 1300.5737), 1299.5621 (100, $[M+H]^+$, $C_{61}H_{75}N_{18}O_{15}^+$; calc. 1299.5659).

Z-Aeg(H)-Aeg(A^Z)-Aeg(G^Z)-Aem · TFA (= N-*(6,9-Dihydro-9-{3-(2-{[2-(morpholin-4-yl)ethyl]-amino}-2-oxoethyl)-2,7,13,19-tetraoxo-21-phenyl-9-[2-(6-{[(phenylmethoxy)carbonyl]amino}-9*H*-purin-9-yl)acetyl]-20-oxa-3,6,9,12,15,18-hexaazaheneicos-1-yl}-6-oxo-1*H*-purin-2-yl)carbamic Acid Phenylmethyl Ester*; **35**). Compound **34** (1.38 g, 1.06 mmol) was treated with TFA (5 ml) according to *GP 1* in the presence of Et_3SiH (1.00 ml, 6.26 mmol). The crude product was taken up in AcOEt (10 ml), triturated with Et_2O, filtered off with suction, washed with Et_2O, and dried to give **35** (1.44 g, 1.01 mmol, 95%). Amorphous solid. *As the product decomposed at 110°, no HT-NMR characterization was possible.* MALDI-MS: 1223.223 (355, $[M+Na]^+$), 1200.790 (877, $[M+2\,H]^+$). HR-ESI-MS: 1202.5178 (6, $[M+4\,H]^+$, $C_{56}H_{70}N_{18}O_{13}^+$; calc. 1202.5369), 1201.5152 (24, $[M+3\,H]^+$, $C_{56}H_{69}N_{18}O_{13}^+$; calc. 1201.5291), 1200.5123 (68, $[M+2\,H]^+$, $C_{56}H_{68}N_{18}O_{13}^+$; calc. 1200.5213), 1199.5062 (100, $[M+H]^+$, $C_{56}H_{67}N_{18}O_{13}^+$; calc. 1199.5135).

Z-Aeg(C^Z)-Aeg(A^Z)-Aeg(G^Z)-Aem · TFA (= N-*(6,9-Dihydro-9-{3-(2-{[2-(morpholin-4-yl)ethyl]-amino}-2-oxoethyl)-2,7,13,19-tetraoxo-15-[2-(2-oxo-4-{[(phenylmethoxy)carbonyl]amino}pyrimidin-1(2*H*)-yl)acetyl]-21-phenyl-9-[2-(6-{[(phenylmethoxy)carbonyl]amino}-9*H*-purin-9-yl)acetyl]-20-oxa-3,6,9,12,15,18-hexaazaheneicos-1-yl}-6-oxo-1*H*-purin-2-yl)carbamic Acid Phenylmethyl Ester*; **36**). Compounds **35** (1.00 g, 0.701 mmol) and *C^Z-AcOH* (**30**; 266 mg, 0.876 mmol) were placed in DMF (5 ml) and coupled according to *GP 4*. After completion of the reaction, the mixture was concentrated *in vacuo* with a dry-ice evaporator. The residue was triturated with ^{i}PrOH, purified by sonication, and the resulting precipitate was collected by suction. The crude product (1.08 g) was purified by semi-prep. RP-HPLC. Freeze drying gave **36** (531 mg, 0.332 mmol, 47%). Colorless powder. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°; rotamers): 8.56, 8.22, 8.21, 8.02 (4*s*, H–C(2) of A, H–C(8) of A); 7.76 (*m*, H–C(6) of C, H–C(8) of G); 7.92–7.17 (*m*, 20 arom. H of Z); 6.88 (*m*, H–C(5) of C); 5.27 (*s*, CH_2 of Z); 5.24 (*s*, CH_2 of Z); 5.20 (*s*, CH_2 of Z); 5.19 (*s*, CH_2 of Z); 5.05 (br. *s*, base CH_2); 5.01 (br. *s*, base CH_2); 4.70 (br. *s*, base CH_2); 3.78–3.10 (*m*, 2 NCH_2CH_2O, 4 CH_2CH_2, 3 CH_2 of Gly). *The occurrence of very broad* s *between 8.10–7.60 and at 6.42 was a result of the diverse NH species and complicated integration.* MALDI-MS: 1508.376 (34, $[M+Na]^+$), 1486.173 (156, $[M+3\,H]^+$). HR-ESI-MS: 1487.5917 (12, $[M+4\,H]^+$, $C_{70}H_{81}N_{21}O_{17}^+$; calc. 1487.6119), 1486.5889 (37, $[M+3\,H]^+$, $C_{70}H_{80}N_{21}O_{17}^+$; calc. 1486.6041), 1485.5863 (87, $[M+2\,H]^+$, $C_{70}H_{79}N_{21}O_{17}^+$; calc. 1485.5962), 1484.5843 (100, $[M+H]^+$, $C_{70}H_{78}N_{21}O_{17}^+$; calc. 1484.5884).

Boc-Aeg(Fmoc)-OBn (= *Benzyl* N-*(2-{[(*tert*-Butoxy)carbonyl]amino}ethyl)*-N-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}glycinate*; **37**). A suspension of **38** (12.0 g, 20.4 mmol; see below) in (420 ml) was cooled to 0° in an ice bath under Ar. EtN^iPr_2 (7.11 ml, 40.8 ml) was then added, followed by the dropwise addition of Boc_2O (4.45 g, 20.4 mmol) in 120 ml of CH_2Cl_2 during 20 min. The mixture was stirred for 1 h at 0° and for a further 4 h at r.t. After cooling to 0°, EtN^iPr_2 (3.55 ml, 20.4 mmol) was added, followed by the addition of Fmoc–Cl (5.28 g, 20.4 mmol). The mixture was stirred overnight and then successively washed with 1M aq. $KHSO_4$, sat. aq. $NaHCO_3$, and brine (250 ml each), dried ($MgSO_4$), and concentrated *in vacuo*. The resulting oil was purified by CC (SiO_2; CH_2Cl_2/MeOH 50:1) to give **37** (7.89 g, 14.9 mmol, 73%). Colorless oil. R_f (DCM/MeOH 50:1) 0.41. ^{1}H-NMR (200 MHz, (D_6)DMSO; 2 rotamers): 7.90 (*d*, *J* = 7.2, 1 arom. H of Fmoc); 7.86 (*d*, *J* = 7.2, 1 arom. H of Fmoc); 7.67 (*d*, *J* = 7.2, 1 arom. H of Fmoc); 7.57 (*d*, *J* = 7.2, 1 arom. H of Fmoc); 7.33 (*m*, 4 arom. H of Fmoc, 5 arom. H of Bn); 6.71 (br. *s*, NH); 5.15 (*s*, 1 H, $PhCH_2$); 5.10 (*s*, 1 H, $PhCH_2$); 4.12 (*m*, CH of Fmoc, CH_2 of Fmoc, CH_2 of Gly); 3.26, 3.06 (2*m*, CH_2CH_2); 1.35 (*s*, *t*-Bu). FAB-MS: 553.3 (16, $[M+Na]^+$), 531.3 (6, $[M+H]^+$), 431.2 (57, $[M-Boc]^+$), 178.1 (100).

H-Aeg(H)-OBn · 2 TsOH (= *Benzyl* N-*(2-Aminoethyl)glycinate*; **38**). BnOH (60 ml) and TsOH (29.4 g, 154 mmol) were added to a suspension of **1** (7.56 g, 64.0 mmol) in toluene (500 ml). The mixture was refluxed (oil bath, 140°) for 6 h and then concentrated *in vacuo* to *ca.* 150 ml. Et_2O (500 ml) was added, and the mixture was kept at −20° overnight. The precipitate was collected by suction, washed with three portions of Et_2O, and dried *in vacuo* to give **38** (32.2 g, 54.7 mmol, 86%). Amorphous solid. ^{1}H-NMR (200 MHz, D_2O): 7.71 (*m*, 4 arom. H of TsOH), 7.39 (*s*, 5 arom. H of Bn); 7.29 (*m*, 4 arom. H of

TsOH); 5.20 (*s*, PhCH_2); 4.06 (*s*, CH_2 of Gly); 3.52 (*m*, CH_2CH_2); 2.32 (*s*, 2 Me, TsOH). ^{13}C-NMR (50 MHz, D_2O): 166.61 (CO); 142.32, 139.64 (2 arom. C of TsOH); 134.43 (arom. C, Bn); 129.45, 128.93, 128.85, 128.49, 125.40 (3 arom. CH of Bn, 2 arom. CH of TsOH); 68.52 (Ph*C*H_2); 47.71, 44.18 ($H_3N^+CH_2CH_2$, CH_2 of Gly); 35.43 ($H_3N^+CH_2$); 20.54 (Me of TsOH). FAB-MS: 231 (8, $[M+Na]^+$), 209.1 (100, $[M+H]^+$), 119.1 (20, $[M-Bn]^+$).

H-Aeg(Fmoc)-OBn · TFA (= *Benzyl* N-*(2-Aminoethyl)*-N-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-glycinate*; **39**). The reaction of **37** (63.6 g, 120 mmol) with TFA (100 ml) in CH_2Cl_2 (100 ml) was performed according to *GP 1*. The crude product was precipitated with Et_2O and collected by suction to give **39** (56.5 g, 104 mmol, 86%). Amorphous solid. ^{1}H-NMR (200 MHz, (D_6)DMSO; 2 rotamers): 7.87 (*m*, 2 arom. H of Fmoc, H_3N^+); 7.61 (*m*, 2 arom. H of Fmoc); 7.35 (*m*, 4 × arom. H of Fmoc, 5 arom. H of Bn); 5.16 (*s*, 0.78 H, PhCH_2); 5.12 (*s*, 1.22 H, PhCH_2); 4.20 (*m*, CH of Fmoc, CH_2 of Fmoc, CH_2 of Gly); 3.49, 2.90 (2*m*, CH_2CH_2). ^{13}C-NMR (50 MHz, (D_6)DMSO; 2 rotamers): 169.65 (*C*OOBn); 169.62 (*C*OOBn); 155.68 (CO of Fmoc); 155.48 (CO of Fmoc); 143.72 (arom. C of Fmoc); 143.62; (arom. C of Fmoc); 140.72 (arom. C of Fmoc); 135.68 (arom. C of Bn); 135.60 (arom. C of Bn); 128.43 (arom. CH of Bn); 128.16 (arom. CH of Bn); 127.92 (arom. CH of Bn); 127.74 (arom. CH of Fmoc); 127.68 (arom. CH of Fmoc); 127.19 (arom. CH of Fmoc); 127.06 (arom. CH of Fmoc); 125.02 (arom. CH of Fmoc); 124.87 (arom. CH of Fmoc); 120.19 (arom. CH of Fmoc); 120.12 (arom. CH of Fmoc); 67.62, 67.29, 66.23, 66.15 (CH_2 of Fmoc, Ph*C*H_2); 49.41, 48.73, 46.40, 45.84, 37.47, 37.13 (CH of Fmoc, CH_2 of Gly, CH_2CH_2). *The signal of one CH_2 of CH_2CH_2 was expected at* ca. *40 ppm and was supposed to be hidden by the signal of DMSO.* FAB-MS: 453.2 (10, $[M+Na]^+$), 431.2 (100, $[M+H]^+$).

Boc-Aeg(Alloc)-Aeg(Fmoc)-OBn (= *Benzyl* N-*(2-{[*N-*(2-{(*tert-*Butoxy)carbonyl]amino}ethyl)*-N-*[(prop-2-en-1-yloxy)carbonyl]glycyl]amino}ethyl)*-N-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}glycinate*; **40**). The coupling of **19** (13.7 g, 45.3 mmol) with **39** (24.7 g, 45.3 mmol) was performed according to *GP 3*. The crude product was purified by CC (SiO_2; cyclohexane/AcOEt 1:2 → 1:5) to yield pure **40** (23.7 g, 32.7 mmol, 73%). Colorless foam. ^{1}H-NMR (250 MHz, (D_6)DMSO, 100°): 7.85 (*d*, *J* = 7.3, 2 arom. H of Fmoc); 7.61 (*d*, *J* = 7.5, 2 arom. H of Fmoc); 7.56 (br. *t*, NH); 7.43–7.27 (*m*, 4 arom. C of Fmoc, 5 arom. H of Bn); 6.35 (br. *t*, NH); 5.89 (*ddt*, *J* = 17, 11, 5.2, CH_2CH=CH_ZH_E); 5.26 (*ddt*, *dq*-like, *J* = 17, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.14 (*ddt*, *dq*-like, *J* = 11, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.14 (*s*, PhCH_2); 4.52 (*dt*, *J* = 5.2, 1.6, CH_2CH=CH_2); 4.36 (*d*, *J* = 6.3, CH_2 of Fmoc); 4.23 (br. *t*, *J* = 6.3, CH of Fmoc); 4.06 (*s*, CH_2 of Gly); 3.82 (*s*, CH_2 of Gly); 3.42–3.06 (*m*, 2 CH_2CH_2); 1.38 (*s*, *t*-Bu). ^{13}C-NMR (63 MHz, (D_6)DMSO, 100°): 168.86 (CO of Gly); 168.33 (CO of Gly); 155.03 (CO of Fmoc, CO of Alloc); 143.32 (arom. C of Fmoc); 140.29 (arom. C of Fmoc); 135.30 (arom. C of Bn); 132.78 (CH_2*C*H=CH_2); 127.78 (arom. CH of Fmoc); 127.42 (arom. CH of Fmoc); 127.17 (arom. *o*-CH of Bn); 127.02 (arom. *m*-CH of Bn); 126.48 (arom. *p*-CH of Bn); 124.30 (arom. CH of Fmoc); 119.38 (arom. CH of Fmoc); 116.08 (CH_2CH=CH_2); 77.24 (Me_3C); 66.71, 65.57, 64.79 (CH_2CH=CH_2, BnCH_2, CH_2 of Fmoc); 50.03, 48.89, 47.66, 46.40, 38.19 (CH of Fmoc, 2 CH_2 of Gly, 2 CH_2CH_2); *signals of the remaining CH_2 groups were located between 50.84 and 35.43 ppm, but could not be separated unambiguously from the baseline under the given conditions* (93 mM sample, 734 scans); 27.72 (Me of Boc). FAB-MS: 737 (100, $[M+Na]^+$), 715.4 (13, $[M+H]^+$), 615.3 (78, $[M-Boc]^+$).

H-Aeg(Alloc)-Aeg(Fmoc)-OBn · TFA (= *Benzyl* N-*[2-({*N-*(2-Aminoethyl)*-N-*[(prop-2-en-1-yloxy)-carbonyl]glycyl}amino)ethyl]*-N-*{[(9 H-fluoren-9-yl)methoxy]carbonyl}glycinate*; **41**). The reaction of **40** (12.8 g, 17.9 mmol) with TFA (30 ml) in CH_2Cl_2 (30 ml) was performed according to *GP 1*. The crude product (13.0 g, 17.9 mmol, 100%) was directly used for the next step. ^{1}H-NMR (200 MHz, (D_6)DMSO, 100°; rotamers): 7.85 (*m*, 2 arom. H of Fmoc, NH); 7.60 (*m*, 2 arom. H of Fmoc); 7.35 (*m*, 4 arom. H of Fmoc, 5 arom. H of Bn); 5.88 (*m*, CH_2CH=CH_ZH_E, NH); 5.22 (*m*, CH_2CH=CH_2); 5.14 (*s*, PhCH_2); 4.54 (*m*, CH_2CH=CH_2); 4.37 (*m*, CH_2 of Fmoc); 4.24 (*m*, CH of Fmoc); 4.06, 3.96 (2*s*, CH_2 of Gly); 3.91, 3.90 (2*s*, CH_2 of Gly); 3.55 (br. *t*, *J* = 6.1, 2 H of 2 CH_2CH_2), 3.30 (*m*, 4 H of 2 CH_2CH_2), 3.05 (br. *t*, *J* = 6.1, 2 H of 2 CH_2CH_2). FAB-MS: 637.3 (20, $[M+Na]^+$), 615.3 (90, $[M+H]^+$), 154 (100).

Boc-Aeg(C^Z)-Aeg(Alloc)-Aeg(Fmoc)-OBn (= *Benzyl* N-*{2-[(*N-*[(Allyloxy)carbonyl]*-N-*{2-[(*N-*{[(4-{[(benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(*2H*)-yl]acetyl}*-N-*(2-{[(*tert-*butoxy)carbonyl]-amino}ethyl)glycyl]amino}ethyl)glycyl]amino}ethyl)*-N-*{[(9*H*-fluoren-9-yl)methoxy)carbonyl}glycinate*; **43**). The coupling of *Boc-Aeg(C^Z)-OH* (**42**; 9.01 g, 17.9 mmol) with **41** (13.0 g, 17.9 mmol) was performed according to *GP 3*. The crude product was purified by CC (SiO_2; AcOEt/MeOH 9:1) to yield **43** (12.8 g,

11.6 mmol, 65%). Colorless foam. R_f (AcOEt/MeOH 9:1) 0.15. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°): 7.78 (*m*, H–C(6) of C, 2 arom. CH of Fmoc, NH); 7.60 (*d*, *J* = 7.4, 2 arom. H of Fmoc), 7.40 (br. *t*, *J* = 5.1, NH); 7.42–7.25 (*m*, 5 arom. H of Z, 5 arom. H of Bn, 4 arom. CH of Fmoc); 6.92 (*d*, *J* = 7.3, H–C(5) of C); 6.37 (br. *s*, NH); 5.89 (*ddt*, *J* = 17, 11, 5.2, CH_2CH=CH_ZH_E); 5.27 (*ddt*, *dq*-like, *J* = 17, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.14 (*ddt*, *dq*-like, *J* = 11, 1.6, 1.6, CH_2CH=CH_ZH_E); 5.21 (*s*, PhCH_2); 5.14 (*s*, PhCH_2); 4.70 (*s*, CH_2–N(1)); 4.51 (*dt*, *J* = 5.2, 1.6, CH_2CH=CH_2); 4.36 (*d*, *J* = 6.0, CH_2 of Fmoc); 4.22 (br. *t*, *J* = 6.0, CH of Fmoc); 4.06 (*s*, CH_2 of Gly); 3.98 (br. *s*, CH_2 of Gly); 3.84 (*s*, CH_2 of Gly); 3.46–3.16 (*m*, 3 CH_2CH_2); 1.40 (*s*, *t*-Bu). FAB-MS: 1122.6 (40, $[M+Na]^+$), 91.1 (100).

Boc-Aeg(C^Z)-Aeg(Alloc)-Aeg(H)-OBn (= *Benzyl* N-*({[2-[(*N-*[(Allyloxy)carbonyl]*-N-*(2-{[(*N-*(4-{[(benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(2*H*)-yl)acetyl]*-N-*(2-{[(*tert-*butoxy)carbonyl]amino}ethyl)glycyl]amino}ethyl)glycyl]amino}ethyl)glycinate*; **44**). Compound **43** (7.40 g, 6.72 mmol) was dissolved in CH_2Cl_2 at r.t. and treated with Et_2NH (10.4 ml, 101 mmol). After stirring for 1.5 h, the solvent was evaporated *in vacuo*, and the residue was triturated with AcOEt (50 ml) and Et_2O (200 ml). The resulting suspension was stirred for 10 min; then, the precipitate was collected by filtration and rinsed with Et_2O to give **44** (5.06 g, 5.77 mmol, 86%). Amorphous solid. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°): 7.82 (*d*, *J* = 7.3, H–C(6) of C); under it (br. *m*, NH); 7.52 (br. *t*, NH); 7.40 (*m*, 5 arom. H of Z, 5 arom. H of Bn); 6.93 (*d*, *J* = 7.3, H–C(5) of C); 6.39 (br. *s*, NH); 5.90 (*ddt*, *J* = 17, 11, 5.2, CH_2CH=CH_ZH_E); 5.33–5.12 (*m*, CH_2CH=CH_2); 5.21 (*s*, PhCH_2); 5.15 (*s*, PhCH_2); 4.71 (*s*, CH_2–N(1)); 4.52 (*dt*, *J* = 5.2, 1.6, CH_2CH=CH_2); 3.98 (*s*, CH_2 of Gly); 3.86 (*s*, CH_2 of Gly); 3.51–3.15 (*m*, 2.5 CH_2CH_2); 3.41 (*s*, CH_2 of Gly); 2.68 (*t*, *J* = 6.3, 0.5 CH_2CH_2); 1.41 (*s*, *t*-Bu). MALDI-MS: 878.940 (128, $[M+H]^+$). HR-ESI-MS: 880.4082 (13, $[M+3\,H]^+$, $C_{42}H_{58}N_9O_{12}^+$; calc. 880.4205), 879.4054 (48, $[M+2\,H]^+$, $C_{42}H_{57}N_9O_{12}^+$; calc. 879.4126), 878.4023 (100, $[M+H]^+$, $C_{42}H_{56}N_9O_{12}^+$; calc. 878.4048).

Boc-Aeg(C^Z)-Aeg(Alloc)-Aeg(G^Z)-OBn (= *Benzyl* N-*[(Allyloxy)carbonyl]*-N-*[(2-{[(benzyloxy)-carbonyl]amino}-6-oxo-1,6-dihydro-9*H*-purin-9-yl)acetyl]*-N-*(2-{[*N-*({2-[(*N-*{[4-{[(benzyloxy)carbonyl]-amino}-2-oxopyrimidin-1(2*H*)-yl]acetyl}*-N-*(2-{[(*tert-*butoxy)carbonyl]amino}ethyl)glycyl]amino}ethyl)-glycyl]amino}ethyl)glycinate*; **45**). The reaction of **44** (4.84 g, 5.51 mmol) with **28** (2.12 g, 6.17 mmol) in DMF (30 ml) was performed according to *GP 4*. The resulting mixture was diluted with AcOEt (300 ml), successively washed with 1M aq. $KHSO_4$, sat. aq. $NaHCO_3$, and brine (250 ml each), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting oil was purified by CC (SiO_2; AcOEt/MeOH 4:1) to give **45** (4.80 g, 3.99 mmol, 72%). Colorless foam. R_f (AcOEt/MeOH 4:1) 0.08. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°; rotamers): 10.76 (br. *s*, 2 NH of Z); 7.80 (*m*, H–C(6) of C, H–C(8) of G, 2 NH); 7.37 (*m*, 10 arom. H of Z, 5 arom. H of Bn); 6.93 (*m*, H–C(5) of C); 6.39 (br. *s*, NH); 5.88 (*m*, CH_2CH=CH_ZH_E); 5.31–5.03 (*m*, CH_2CH=CH_2, PhCH_2, CH_2–N(9) of G); 5.27 (*s*, PhCH_2); 5.21 (*s*, PhCH_2); 4.71 (*s*, CH_2–N(1) of C); 4.50 (*m*, CH_2CH=CH_2); 4.24 (*s*, CH_2 of Gly); 3.98 (*s*, CH_2 of Gly); 3.89 (*s*, CH_2 of Gly); 3.55–3.19 (*m*, 3 CH_2CH_2); 1.40 (*s*, *t*-Bu). FAB-MS: 1203.5 (8, $[M+H^+]$, 91.1 (100).

Boc-Aeg(C^Z)-Aeg(H)-Aeg(G^Z)-OBn (= *Benzyl* N-*[(2-{[(Benzyloxy)carbonyl]amino}-6-oxo-1,6-dihydro-9*H*-purin-9-yl)acetyl]*-N-*(2-{[*N-*(2-[(*N-*{[4-{[(benzyloxy)carbonyl]amino}-2-oxopyrimidin-1-(2*H*)-yl]acetyl}*-N-*{2-{[(*tert-*butoxy)carbony]amino}ethyl)glycyl]amino}ethyl)glycyl]amino}ethyl)glycinate*; **46**). Compound **45** (5.17 g, 4.30 mmol) was dissolved in dry THF, followed by the addition of NDMBA (15.4 g, 98.8 mmol) and $Pd[P(Ph)_3]_4$ (991 mg, 0.858 mmol) one after another. After stirring at r.t. for 2 h, the solvent was evaporated *in vacuo*, and the residue was purified by CC (SiO_2; AcOEt/MeOH 2:1 → 1:1) to give **46** (4.21 g, 3.76 mmol, 88%). Colorless foam. R_f (AcOEt/MeOH) 0.28. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°; rotamers): 7.77 (*m*, H–C(6) of C, H–C(8) of G, 2 NH); 7.33 (*m*, 10 arom. of Z, 5 arom. H of Bn); 6.91 (*m*, H–C(5) of C); 6.39 (br. *s*, NH); 5.88 (*m*, CH_2CH=CH_ZH_E); 5.31–5.03 (*m*, CH_2CH=CH_2, PhCH_2, CH_2–N(9) of G); 5.27 (*s*, PhCH_2); 5.21 (*s*, PhCH_2); 4.71 (*s*, CH_2–N(1) of C); 4.50 (*m*, CH_2CH=CH_2); 4.24 (*s*, CH_2 of Gly); 3.98 (*s*, CH_2 of Gly); 3.89 (*s*, CH_2 of Gly); 3.55–3.19 (*m*, 3 CH_2CH_2); 1.40 (*s*, *t*-Bu). MALDI-MS: 1143.260 (317, $[M+Na]^+$), 1120.603 (1053, $[M+2\,H]^+$). HR-ESI-MS: 1122.4695 (5, $[M+4\,H]^+$, $C_{53}H_{66}N_{14}O_{14}^+$; calc. 1122.4883), 1121.46703 (22, $[M+3\,H]^+$, $C_{53}H_{65}N_{14}O_{14}^+$; calc. 1121.4804), 1120.4646 (61, $[M+2\,H]^+$, $C_{53}H_{64}N_{14}O_{14}^+$; calc. 1120.4726), 1119.4620 (100, $[M+H]^+$, $C_{53}H_{63}N_{14}O_{14}^+$; calc. 1119.4648).

Boc-Aeg(C^Z)-Aeg(F)-Aeg(G^Z)-OBn (= *Benzyl* N-*[2-{[(Benzyloxy)carbonyl]amino}-6-oxo-1,6-dihydro-9*H*-purin-9-yl)acetyl]*-N-*[2-({[(*N-*{2-[(*N-*{[4-{[(benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(2*H*)-yl]acetyl}*-N-*(2-{[(*tert-*butoxy)carbonyl]amino}ethyl)glycyl]amino}ethyl]*-N-*[(2,4-difluoro-5-

methylphenyl)acetyl]glycyl}amino)ethyl]glycinate; **47**). The coupling of **2** (300 mg, 1.61 mmol) with **46** (1.50 g, 1.34 mmol) was performed according to *GP 3*. The resulting mixture was diluted with AcOEt (200 ml), successively washed with 1M aq. $KHSO_4$, sat. aq. $NaHCO_3$, and brine (200 ml each), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting oil was purified by CC (SiO_2; AcOEt/MeOH 2:1) to give **47** (1.29 g, 1.01 mmol, 75%). Colorless foam. R_f (AcOEt/MeOH 2:1) 0.24. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°; rotamers): 7.97–7.72 (*m*, 5 H), 7.47–7.10 (*m*, 15 H), 6.92–6.81 (*m*, 2 H) (H–C(6) of C, H–C(5) of C, H–C(8) of G, H–C(6) of F, H–C(3) of F, 10 arom. H of Z, 5 arom. H of Bn, 2 NH); 6.42 (br. *s*, NH); 5.25, 5.20, 5.17, 5.03, 4.92 (5*s*, 3 PhCH_2); 4.73 (*m*, base CH_2); 4.10 (*m*, 2 base CH_2); 3.65–2.76 (*m*, 3 CH_2 of Gly, 3 CH_2CH_2) 1.98 (br. *s*, Me of F); 1.39 (*m*, *t*-Bu). ^{19}F-NMR (235 MHz, (D_6)DMSO; rotamers): −119.63 (*m*, F–C(4)); −120.14 (*m*, F–C(2)). FAB-MS: 1309.3 (100, [*M* + Na]$^+$).

H-Aeg(C^Z)-Aeg(F)-Aeg(G^Z)-OBn · TFA (= *Benzyl* N-*[2-({*N-*(2-{[*N-*(2-Aminoethyl)*-N-*{[4-{[(benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(2*H*)-yl]acetyl}glycyl]amino}ethyl)*-N-*[(2,4-difluoro-5-methylphenyl)acetyl]glycyl}amino)ethyl]*-N-*[(2-{[(benzyloxy)carbonyl]amino}-6-oxo-1,6-dihydro-9*H*-purin-9-yl)acetyl]glycinate*; **48**). Compound **47** (1.43 g, 1.11 mmol) was treated with TFA (5 ml) and Et_3SiH (2.5 ml, 15.7 mmol) according to *GP 1*. The crude product was precipitated with Et_2O and collected by suction to give **48** (1.27 g, 0.975 mmol, 88%). Amorphous solid. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°; rotamers): 8.08 (br. *s*, NH); 7.95–7.68 (*m*, 4 H), 7.40–7.34 (*m*, 15 H), 7.11 (br. *s*, 1 H), 6.93–6.82 (*m*, 2 H) (H–C(6) of C, H–C(5) of C, H–C(8) of G, H–C(6) of F, H–C(3) of F, 10 arom. H of Z, 5 arom. H of Bn, NH); 6.42 (br. *s*, NH); 5.27, 5.21, 5.18, 5.02 (4*s*, 3 PhCH_2); 4.80–4.60 (*m*, base CH_2); 4.26 (br. *s*, 2 H), 4.08 (br. *m*, 4 H); 3.64–3.09 (br. *m*, 14 H) (2 base CH_2, 3 CH_2 of Gly, 3 CH_2CH_2); 1.98 (br. *s*, Me of F). ^{19}F-NMR (235 MHz, (D_6)DMSO; rotamers): −77.01 (*s*, F_3CCOOH); −119.40, −120.12 (2*m*, F–C(2), F–C(4)). MALDI-MS: 1188.331 (70, [*M* + 2 H]$^+$). HR-ESI-MS: 1190.4525 (8, [*M* + 4 H]$^+$, $C_{57}H_{64}F_2N_{14}O_{13}^+$; calc. 1190.4745), 1189.4532 (24, [*M* + 3 H]$^+$, $C_{57}H_{63}F_2N_{14}O_{13}^+$; calc. 1189.4667), 1188.4504 (67, [*M* + 2 H]$^+$, $C_{57}H_{62}F_2N_{14}O_{13}^+$; calc. 1188.4589), 1187.4474 (100, [*M* + H]$^+$, $C_{57}H_{61}F_2N_{14}O_{13}^+$; calc. 1187.4510).

Me$_2$-Aeg(Me)-Aeg(C^Z)-Aeg(F)-Aeg(G^Z)-OBn · 2 TFA (= *Benzyl* N-*[(2-{[(Benzyloxy)carbonyl]amino}-6-oxo-1,6-dihydro-9*H*-purin-9-yl)acetyl]*-N-*{2-[(*N-*{2-[(*N-*{[4-{[(benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(2*H*)-yl]acetyl}*-N-*[2-({*N-*[2-(dimethylamino)ethyl]*-N-*methylglycyl}amino)ethyl]glycyl)-N-[(2,4-difluoro-5-methylphenyl)acetyl]glycyl)amino]ethyl}glycinate*; **49**). Compounds **5a** and **48** were coupled as described for the preparation of **27**. The crude product was purified by RP-HPLC to give **49** (305 mg, 214 mmol, 46%). Colorless powder. ^{1}H-NMR (200 MHz, (D_6)DMSO, 110°): 7.76 (*m*, H–C(6) of C, arom. H of F, H–C(8) of G, 2 NH); 7.41–7.34 (*m*, 10 arom. H of Z, 5 arom. H of Bn); 7.12 (br. *t*, NH); 6.89 (*m*, H–C(5) of C, arom. H of F); 5.27 (*s*, PhCH_2); 5.21 (*s*, PhCH_2); 5.18 (br. *s*, PhCH_2); 5.02 (br. *s*, base CH_2); 4.69 (br. *s*, base CH_2); 4.24 (br. *s*, base CH_2); 4.02 (br. *s*, 2 CH_2 of Gly); 3.63–3.20 (br. *m*, 2 CH_2 of Gly, 3.5 CH_2CH_2); 2.90 (*t*, *J* = 6.0, 0.5 CH_2CH_2); 2.84 (*s*, Me_2N); 2.40 (*s*, MeN); 2.12 (br. *s*, Me of F). ^{19}F-NMR (235 MHz, (D_6)DMSO; rotamers): −77.83 (*s*, 2 F_3CCOOH); −119.39, −120.09 (2*m*, F–C(2), F–C(4)). MALDI-MS: 1330.842 (577, [*M* + 2 H]$^+$. HR-ESI-MS: 1332.5663 (8, [*M* + 4 H]$^+$, $C_{64}H_{78}F_2N_{16}O_{14}^+$; calc. 1332.5851), 1331.5630 (24, [*M* + 3 H]$^+$, $C_{64}H_{77}F_2N_{16}O_{14}^+$; calc. 1331.5773), 1330.5610 (73, [*M* + 2 H]$^+$, $C_{64}H_{76}F_2N_{16}O_{14}^+$; calc. 1330.5695), 1329.5583 (100, [*M* + H]$^+$, $C_{64}H_{75}F_2N_{16}O_{14}^+$; calc. 1329.5616).

REFERENCES

[1] Y. L. Jiang, L. M. McDowell, B. Poliks, D. R. Studelska, C. Cao, G. S. Potter, J. Schaefer, F. Song, J. T. Stivers, *Biochemistry* **2004**, *43*, 15429.

[2] C. Kreutz, H. Kählig, R. Konrat, R. Micura, *J. Am. Chem. Soc.* **2005**, *127*, 11558.

[3] G. L. Olsen, T. E. Edwards, P. Deka, G. Varani, S. T. Sigurdsson, G. P. Drobny, *Nucleic Acids Res.* **2005**, *33*, 3447.

[4] C. Kreutz, H. Kählig, R. Konrat, R. Micura, *Angew. Chem., Int. Ed.* **2006**, *45*, 3450.

[5] M. Hennig, M. L. Munzarová, W. Bermel, L. G. Scott, V. Sklenář, J. R. Williamson, *J. Am. Chem. Soc.* **2006**, *128*, 5851.

[6] M. Hennig, L. G. Scott, E. Sperling, W. Bermel, J. R. Williamson, *J. Am. Chem. Soc.* **2007**, *129*, 14911.
[7] N. B. Barhate, R. N. Barhate, P. Cekan, G. Drobny, S. T. Sigurdsson, *Org. Lett.* **2008**, *10*, 2745.
[8] D. Graber, H. Moroder, R. Micura, *J. Am. Chem. Soc.* **2008**, *130*, 17230.
[9] D. A. Pfaff, K. M. Clarke, T. A. Parr, J. M. Cole, B. H. Geierstanger, D. C. Tahmassebi, T. J. Dwyer, *J. Am. Chem. Soc.* **2008**, *130*, 4869.
[10] B. Puffer, C. Kreutz, U. Rieder, M.-O. Ebert, R. Konrat, R. Micura, *Nucleic Acids Res.* **2009**, *37*, 7728.
[11] P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **1991**, *254*, 1497.
[12] N. Shibata, B. K. Das, H. Honjo, Y. Takeuchi, *J. Chem. Soc., Perkin Trans. 1* **2001**, 1605.
[13] K. A. Frey, S. A. Woski, *Chem. Commun.* **2002**, 2206.
[14] M. Hollenstein, C. J. Leumann, *Org. Lett.* **2003**, *5*, 1987.
[15] M. Hollenstein, C. J. Leumann, *J. Org. Chem.* **2005**, *70*, 3205.
[16] B. Kuhnast, F. Dolle, B. Tavitian, *J. Labelled Compd. Radiopharm.* **2002**, *45*, 1.
[17] P. E. Nielsen, *Origins Life Evol. Biosphere* **1993**, *23*, 323.
[18] P. E. Nielsen, *Chem. Biodiversity* **2007**, *4*, 1996.
[19] P. E. Nielsen, *Origins Life Evol. Biosphere* **2007**, *37*, 323.
[20] G. K. Mittapalli, Y. M. Osornio, M. A. Guerrero, K. R. Reddy, R. Krishnamurthy, A. Eschenmoser, *Angew. Chem., Int. Ed.* **2007**, *46*, 2478.
[21] G. K. Mittapalli, K. R. Reddy, H. Xiong, O. Munoz, B. Han, F. De Riccardis, R. Krishnamurthy, A. Eschenmoser, *Angew. Chem., Int. Ed.* **2007**, *46*, 2470.
[22] A. Eschenmoser, *Tetrahedron* **2007**, *63*, 12821.
[23] K. E. Nelson, M. Levy, S. L. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3868.
[24] S. Rasmussen, L. Chen, M. Nilsson, S. Abe, *Artificial Life* **2003**, *9*, 269.
[25] S. Rasmussen, L. Chen, D. Deamer, D. C. Krakauer, N. H. Packard, P. F. Stadler, M. A. Bedau, *Science* **2004**, *303*, 963.
[26] T. Rocheleau, S. Rasmussen, P. E. Nielsen, M. N. Jacobi, H. Ziock, *Philos. Trans. R. Soc., London, Ser. B* **2007**, *362*, 1841.
[27] 'Protocells – Bridging Nonliving and Living Matter', Eds. S. Rasmussen, M. A. Bedau, L. Chen, D. Deamer, D. C. Krakauer, N. H. Packard, P. F. Stadler, MIT Press, Cambridge, London, 2009.
[28] C. Böhler, P. E. Nielsen, L. E. Orgel, *Nature* **1995**, *376*, 578.
[29] J. G. Schmidt, L. Christensen, P. E. Nielsen, L. E. Orgel, *Nucleic Acids Res.* **1997**, *25*, 4792.
[30] J. G. Schmidt, P. E. Nielsen, L. E. Orgel, *J. Am. Chem. Soc.* **1997**, *119*, 1494.
[31] J. G. Schmidt, P. E. Nielsen, L. E. Orgel, *Nucleic Acids Res.* **1997**, *25*, 4797.
[32] M. Koppitz, P. E. Nielsen, L. E. Orgel, *J. Am. Chem. Soc.* **1998**, *120*, 4563.
[33] A. Mattes, O. Seitz, *Chem. Commun.* **2001**, 2050.
[34] A. Mattes, O. Seitz, *Angew. Chem., Int. Ed.* **2001**, *40*, 3178.
[35] D. M. Rosenbaum, D. R. Liu, *J. Am. Chem. Soc.* **2003**, *125*, 13924.
[36] R. E. Kleiner, Y. Brudno, M. E. Birnbaum, D. R. Liu, *J. Am. Chem. Soc.* **2008**, *130*, 4646.
[37] J. M. Heemstra, D. R. Liu, *J. Am. Chem. Soc.* **2009**, *131*, 11347.
[38] I. Stahl, G. von Kiedrowski, *J. Am. Chem. Soc.* **2006**, *128*, 14014.
[39] B. A. Schweitzer, E. T. Kool, *J. Org. Chem.* **1994**, *59*, 7238.
[40] E. T. Kool, H. O. Sintim, *Chem. Commun.* **2006**, 3665.
[41] A. W. van der Made, R. H. van der Made, *J. Org. Chem.* **1993**, *58*, 1262.
[42] P. Wittung, P. E. Nielsen, O. Buchardt, M. Egholm, B. Nordén, *Nature* **1994**, *368*, 561.
[43] P. Sazani, S.-H. Kang, M. A. Maier, C. Wei, J. Dillman, J. Summerton, M. Manoharan, R. Kole, *Nucleic Acids Res.* **2001**, *29*, 3965.
[44] B. P. Gangamani, V. A. Kumar, K. N. Ganesh, *Biochem. Biophys. Res. Commun.* **1997**, *240*, 778.
[45] B. Hyrup, M. Egholm, O. Buchardt, P. E. Nielsen, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1083.
[46] P. E. Nielsen, G. Haaima, A. Lohse, O. Buchardt, *Angew. Chem., Int. Ed.* **1996**, *35*, 1939.
[47] E. Lesnik, F. Hassman, J. Barbeau, K. Teng, K. Weiler, R. Griffey, S. Freier, *Nucleosides Nucleotides* **1997**, *16*, 1775.
[48] F. Bergmann, W. Bannwarth, S. Tam, *Tetrahedron Lett.* **1995**, *36*, 6823.
[49] E. Uhlmann, D. W. Will, G. Breipohl, D. Langner, A. Ryte, *Angew. Chem., Int. Ed.* **1996**, *35*, 2632.
[50] L. Good, S. K. Awasthi, R. Dryselius, O. Larsson, P. E. Nielsen, *Nat. Biotechnol.* **2001**, *19*, 360.

[51] C. G. Simmons, A. E. Pitts, L. D. Mayfield, J. W. Shay, D. R. Corey, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3001.
[52] G. M. Bonora, S. Drioli, M. Ballico, A. Faccini, R. Corradini, S. Cogoi, L. Xodo, *Nucleosides, Nucleotides Nucleic Acids* **2007**, *26*, 661.
[53] P. R. Berthold, T. Shiraishi, P. E. Nielsen, *Bioconjugate Chem.* **2010**, *21*, 1933.
[54] B. D. Gildea, S. Casey, J. MacNeill, H. Perry-O'Keefe, D. Sørensen, J. M. Coull, *Tetrahedron Lett.* **1998**, *39*, 7255.
[55] G. von Kiedrowski, *Angew. Chem., Int. Ed.* **1986**, *25*, 932.
[56] G. von Kiedrowski, B. Wlotzka, J. Helbing, *Angew. Chem., Int. Ed.* **1989**, *28*, 1235.
[57] G. von Kiedrowski, B. Wlotzka, J. Helbing, M. Matzen, S. Jordan, *Angew. Chem., Int. Ed.* **1991**, *30*, 423.
[58] H. Rasmussen, J. Sandholm Kastrup, J. N. Nielsen, J. M. Nielsen, P. E. Nielsen, *Nat. Struct. Biol.* **1997**, *4*, 98.
[59] G. von Kiedrowski, 'Bioorganic Chemistry Frontiers', Vol. 3, Springer Verlag, 1993, p. 113.
[60] J. M. Coull, M. Egholm, R. P. Hodge, M. Ismail, S. B. Rajur, PCT Int. Appl. WO 96/40685, 1996.
[61] L. Christensen, R. Fitzpatrick, B. Gildea, K. H. Petersen, H. F. Hansen, T. Koch, M. Egholm, O. Buchardt, P. E. Nielsen, J. Coull, R. H. Berg, *J. Pept. Sci.* **1995**, *1*, 175.
[62] G. Breipohl, J. Knolle, D. Langner, G. O'Malley, E. Uhlmann, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 665.
[63] S. A. Thomson, J. A. Josey, R. Cadilla, M. D. Gaul, C. F. Hassman, M. J. Luzzio, A. J. Pipe, K. L. Reed, D. J. Ricca, R. W. Wiethe, S. A. Noble, *Tetrahedron* **1995**, *51*, 6179.
[64] A. Farèse, N. Patino, R. Condom, S. Dalleu, R. Guedj, *Tetrahedron Lett.* **1996**, *37*, 1413.
[65] A. Farese, S. Pairot, N. Patino, V. Ravily, R. Condom, A. Aumelas, R. Guedj, *Nucleosides Nucleotides* **1997**, *16*, 1893.
[66] C. Di Giorgio, S. Pairot, C. Schwergold, N. Patino, R. Condom, A. Farese-Di Giorgio, R. Guedj, *Tetrahedron* **1999**, *55*, 1937.
[67] G. Depecker, C. Schwergold, C. Di Giorgio, N. Patino, R. Condom, *Tetrahedron Lett.* **2001**, *42*, 8303.
[68] M.-L. Leroux, C. Di Giorgio, N. Patino, R. Condom, *Tetrahedron Lett.* **2002**, *43*, 1641.
[69] C. Schwergold, G. Depecker, C. Di Giorgio, N. Patino, F. Jossinet, B. Ehresmann, R. Terreux, D. Cabrol-Bass, R. Condom, *Tetrahedron* **2002**, *58*, 5675.
[70] G. Depecker, N. Patino, C. Di Giorgio, R. Terreux, D. Cabrol-Bass, C. Bailly, A.-M. Aubertin, R. Condom, *Org. Biomol. Chem.* **2004**, *2*, 74.
[71] S. Caldarelli, G. Depecker, N. Patino, A. Di Giorgio, T. Barouillet, A. Doglio, R. Condom, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4435.
[72] E. P. Heimer, H. E. Gallotorres, A. M. Felix, M. Ahmad, T. J. Lambros, F. Scheidl, J. Meienhofer, *Int. J. Pept. Protein Res.* **1984**, *23*, 203.
[73] F. Debaene, N. Winssinger, *Org. Lett.* **2003**, *5*, 4445.
[74] F. Guibé, *Tetrahedron* **1998**, *54*, 2967.
[75] L. Betts, J. A. Josey, J. M. Veal, S. R. Jordan, *Science* **1995**, *270*, 1838.
[76] M. Leijon, A. Graeslund, P. E. Nielsen, O. Buchardt, B. Norden, S. M. Kristensen, M. Eriksson, *Biochemistry* **1994**, *33*, 9820.
[77] S. C. Brown, S. A. Thomson, J. M. Veal, D. G. Davis, *Science* **1994**, *265*, 777.
[78] S.-M. Chen, V. Mohan, J. S. Kiely, M. C. Griffith, R. H. Griffey, *Tetrahedron Lett.* **1994**, *35*, 5105.
[79] T. Vilaivan, C. Suparpprom, P. Harnyuttanakorn, G. Lowe, *Tetrahedron Lett.* **2001**, *42*, 5533.
[80] T. Vilaivan, G. Lowe, *J. Am. Chem. Soc.* **2002**, *124*, 9326.
[81] C. Suparpprom, C. Srisuwannaket, P. Sangvanich, T. Vilaivan, *Tetrahedron Lett.* **2005**, *46*, 2833.
[82] V. A. Kumar, K. N. Ganesh, *Acc. Chem. Res.* **2005**, *38*, 404.

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