Synthesis of Amino Acids with Unnatural Nucleobases or Chromophores Suitable for Use in Model Electron-Transfer Studies

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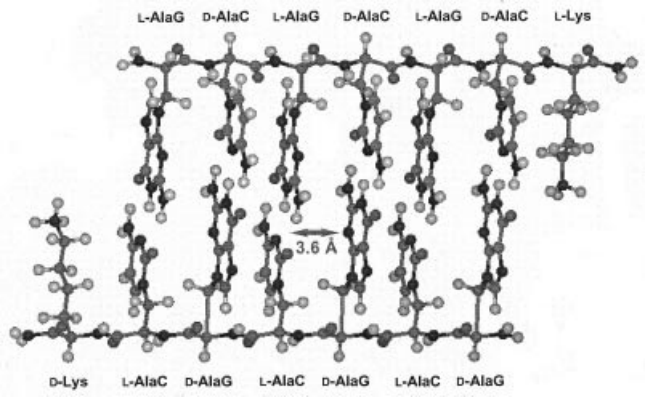
We describe the syntheses of Boc-protected amino acids bearing the chromophores pyromellitic diimide, naphthalene diimide, and pyrene in their side chains, as well as nucleo amino acids with the artificial purines 7-carbaguanine, 7-carbaadenine, isoadenine, and 7-carbaisoadenine covalently linked to the alanyl side chain. These amino acids incorporating chromophores are suitable building blocks for incorporation into alanyl-PNA for future investigations of the mechanism of nucleobase-mediated electron transfer.

Introduction

Electron transfer and hole transfer mediated by the nucleobase stack of a DNA double strand has developed to a lively research area during the last decade.[1] Mechanistic details, however, regarding electron transfer, hole transfer, and charge migration still need to be evaluated.[2] An experimental drawback for electron transfer studies in DNA double strands results from the helix topology. A DNA duplex may unwind or change its conformation when intercalators are bound, or when constitutional changes are required to create an excess charge, electron or hole within the base stack. Therefore, it is quite difficult to predict the base-pair orientation within a perturbed DNA base stack.

In this context, we have introduced alanyl peptide nucleic acids (alanyl-PNA) as model systems for DNA base stacks (Scheme 1).[3] An alanyl-PNA single strand is based on a regular peptide backbone having an alternating configuration of alanyl amino acids. Nucleobases are covalently bound to the alanyl side chains. These artificial oligomers form linear double strands with high rigidity and very well defined base-pair orientations. The linearity of the double strands allows the ability to specifically generate unnatural base pairs and different pairing modes.[4] Furthermore, the orientation of the base-pair stacking can be varied by side-chain homology, whereas the distance between the base pairs can by influenced by the backbone homology.[5] The base stack of alanyl-PNA can be addressed by intercalating



Scheme 1. Linear alanyl peptide nucleic acid double strand as a model system for a DNA nucleobase stack

chromophores[6] or by chromophores covalently linked to the amino acid side chains.[7] In both cases, a site lacking a base pair is required, since alanyl-PNA is already linear and cannot unwind any further. In contrast to DNA, contact between a chromophore and the nucleobase stack in alanyl-PNA proceeds regioselectively without conformational reorganization. This feature makes alanyl-PNA especially valuable as a DNA base-stack model for investigating electron transfer. Alanyl-PNA donor and acceptor chromophores having various oxidation potentials are needed to perform electron-transfer studies with this model system. Furthermore, there is interest in incorporating modified purines next to the canonical nucleobases to allow mediation of the redox potential within the alanyl-PNA base stacks.

Synthesis of Alanyl Amino Acids with Covalently Bound Chromophores

For initial electron-transfer studies in alanyl-PNA, the chromophores 9-amino-6-chloro-2-methoxyacridine and

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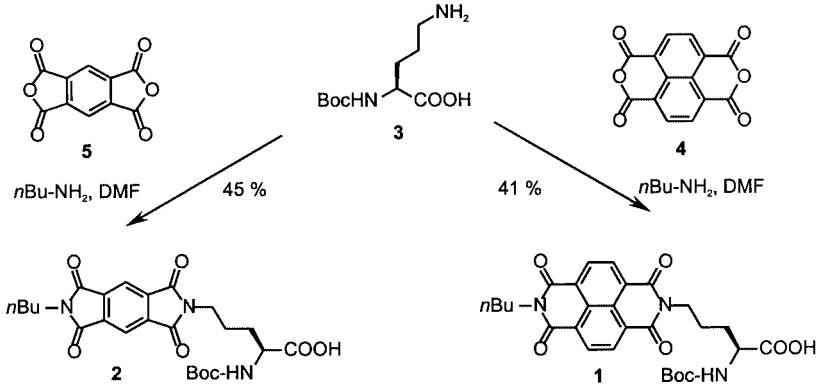


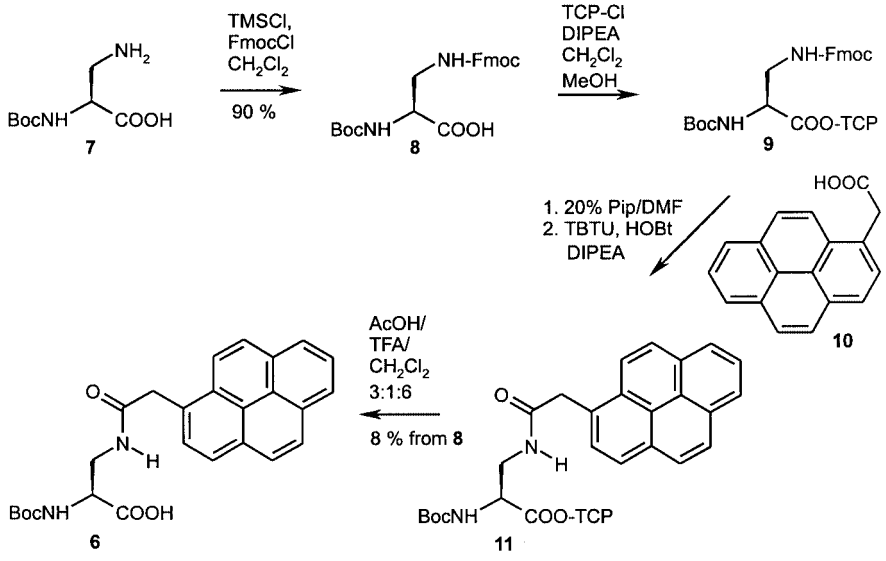
Scheme 2. Synthesis of amino acids having naphthalene diimide and pyromellitic diimide chromophores starting from Boc-ornithine

ethidium were covalently linked to the alanyl side chain[7] and incorporated into alanyl-PNA double strands. In the acridine-modified alanyl-PNA oligomers, there is clear evidence for electron transfer with a distance dependency similar to that found in DNA double strands.[8]

The amino acid with a naphthalene diimide chromophore **1** and amino acid **2** carrying a pyromellitic diimide dye (Scheme 2) were synthesized to function as electron traps in PNA, since their reduction potentials are lower than any of the native nucleobases. In addition, both **1** and **2** are expected to undergo photoexcited hole-transfer reactions with guanine and adenine.[9] The synthesis of amino acid **1** was undertaken by simultaneous reaction of N^{α}-*tert*-butoxycarbonyl (Boc)-protected ornithine (**3**) and *n*-butylamine with naphthalenetetracarboxylic bisanhydride (**4**). Derivative **2** was obtained in an analogous manner by reaction of pyromellitic dianhydride (**5**) with Boc-Orn-OH and *n*-butylamine.

We are also interested in the pyrene derivative **6** (Scheme 3) since it should serve as an electron donor for both cytosine and thymine within a nucleobase stack. Initial attempts to synthesize amino acid **6** by amide-bond formation between *N*-Boc-aminoalanine (**7**) and pyreneacetic acid (**10**) in DMF were hampered because of the poor solubility and aggregation of the respective amino acid derivatives.[10] Therefore, we decided to link the amino acid to a trityl-chloro-polystyrene (TCP) solid support prior to the introduction of the pyrene residue.

N^{α}-Boc-aminoalanine (**7**) was Fmoc protected at the side chain to allow binding of the amino acid **8**[11] as an ester to a TCP-Cl resin. After Fmoc deprotection, amide coupling of the resin-bound aminoalanine **9** to pyreneacetic acid **10** was effected using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) as the coupling reagent, 1-hydroxybenzotriazole (HOBt) as the coupling auxiliary, *N*,*N*-diisopropylethylamine (DIPEA) as the base, and *N*-methyl-2-pyrrolidone (NMP) as the solvent. For purification, it was sufficient merely to wash the resin-bound derivative **11**. The desired amino acid **6** was obtained



Scheme 3. Solid-supported synthesis of a Boc-protected pyrene-appended amino acid

after acidic cleavage from the solid support, followed by evaporation of the solvent.

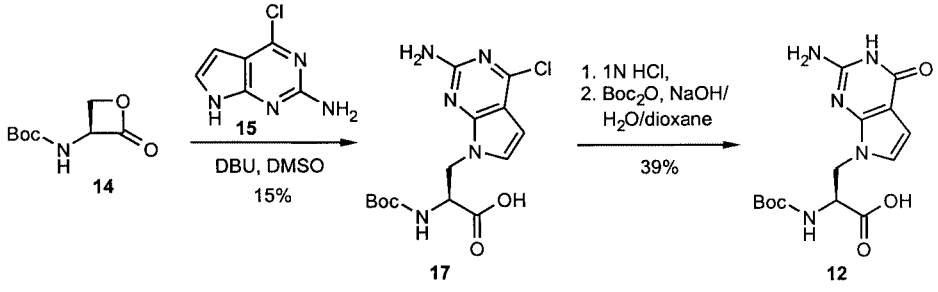
Synthesis of Alanyl Nucleo Amino Acids having Modified Nucleobases

7-Carbaguanine and 7-carbaadenine are better electron donors than the respective guanine and adenine nucleobases.[12] Since regular hydrogen bonding in the Watson−Crick mode is still possible with the 7-deaza derivatives, both these nucleobases can be incorporated into double strands without changing the overall pairing geometry. For incorporation in alanyl-PNA, the Boc-protected nucleo amino acids **12** (Scheme 4) and **13** (Scheme 5) were required. Stereoselective generation of Boc-alanyl nucleo amino acids usually is performed by nucleophilic ring opening of Boc-serine lactone (**14**) with the respective nucleobase.[13] Since both 7-carbaguanine and 7-carbaadenine turned out to be unsuitable nucleophiles, the 6-chloropurine derivatives **15** and **16** were used instead, with subsequent steps needed to generate the desired carboxyl and amino groups. 2-Amino-7-carba-6-chloropurine (**15**) and 7-carba-6-chloropurine (**16**) were prepared by analogy to the procedures of Davoll[14] and Seela[15].

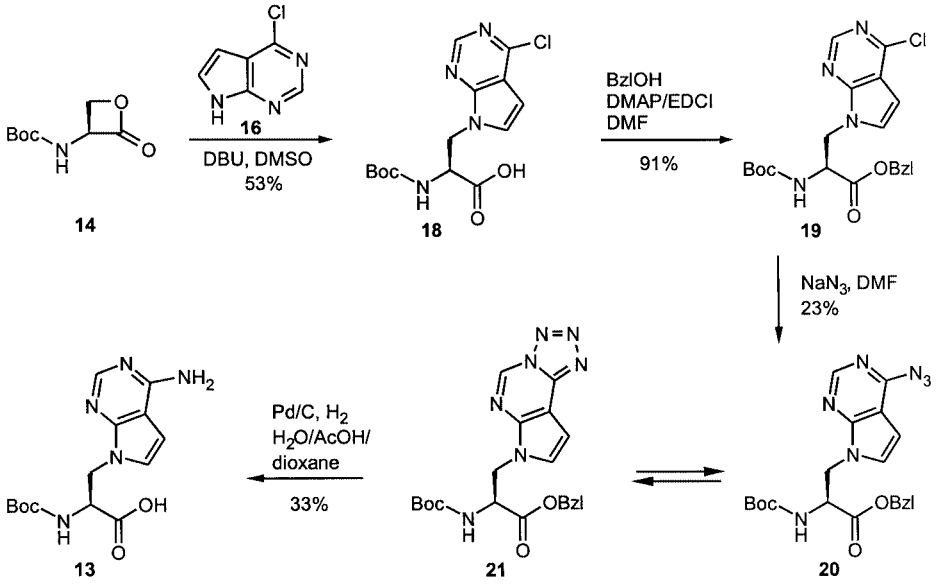
The ring opening of serine lactone **14** was quite difficult, even with 2-amino-6-chloro-7-carbapurine (**15**), and succeeded only with modest yield (Scheme 4). The synthesis of the carbaguaninylalanine **12** was completed by acidic substitution of the purinyl chlorine atom in nucleo amino acid **17**, followed by selective Boc-reprotection of the α-amino group.

6-Chloro7-carbapurine (**16**) turned out to be a good nucleophile for serine lactone **14** to yield the desired nucleo amino acid **18** (Scheme 5). Purine modification by exchange of the chlorine atom into an amino group was performed in three steps — esterification, introduction of the azide, and subsequent one-pot reduction and saponification — to yield the carbaadeninyl nucleo amino acid **13**. Without esterification, reduction of the azide was unsuccessful. Purinyl azides are known to exist in a solvent-dependent equilibrium between the azide and the tetrazole forms.[16] In the case of the carbapurinyl derivative having a free carboxylic acid functionality, only the azide form was detected by NMR spectroscopy in [D_6]DMSO. Therefore, it seems likely that only the tetrazole form **21** was reduced under the hydrogenolytic conditions. Esterification allowed the use of a less polar solvent mixture, which shifts the equilibrium between azide **20** and tetrazole **21** (3:97 in $CDCl_3$) and, consequently, facilitates the reduction.

Finally, the isoadeninyl nucleo amino acid **22** was obtained by catalytic hydrogenolysis of the 6-chloro-2-amino-

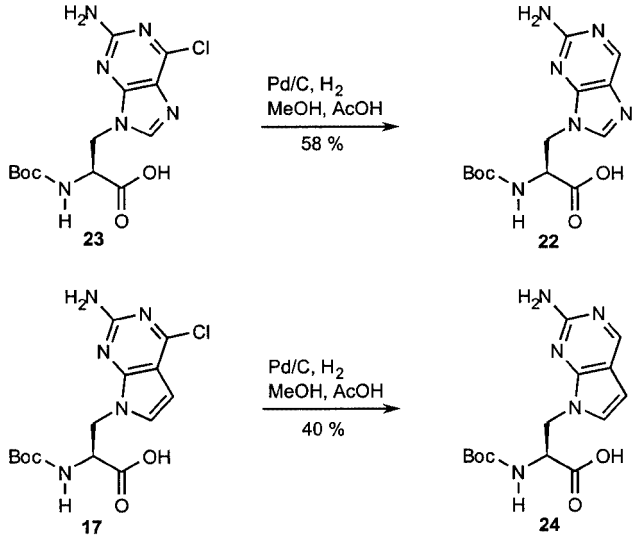


Scheme 4. Synthesis of the N7-carbaguaninyl nucleo amino acid **12** by ring opening of Boc-serine lactone



Scheme 5. Synthesis of N7-carbaadeninyl nucleo amino acid

purinyl derivative **23**[17] (Scheme 6), which is an intermediate in the guaninyl nucleo amino acid synthesis.[3b] In an analogous procedure, the corresponding 7-carbapurinyl derivative **17** was converted into the 7-carbaisoadeninyl nucleo amino acid **24**.[18] For this reaction to proceed well, it was crucial to avoid further reduction, which happens readily at increased temperatures or with higher amounts of catalyst.



Scheme 6. Synthesis of isoadeninyl and N7-carbaisoadeninyl nucleo amino acids by dechlorination of 2-amino-6-chloropurinyl nucleo amino acids

Conclusions

The synthesis of Boc-protected amino acids carrying the chromophores naphthalene diimide, pyromellitic diimide, and pyrene, or the modified nucleobases 7-carbaguanine, 7-carbaadenine, isoadenine, and 7-carbaisoadenine, was performed to allow their incorporation into alanyl-PNA by solid-phase peptide synthesis. Since this set of chromophores provides a wide spectrum of redox potentials, it should be possible for oxidative or reductive interactions with the nucleobase stack of alanyl-PNA double strands to be initiated by the chromophores' excited states. In the special case of alanyl-PNA duplexes, charge-transfer experiments profit from this well-defined and rigid DNA model system. In particular, the conformational reorganization in DNA that results from stacking of a chromophore with the nucleobases can be avoided in alanyl-PNA. The corresponding electron-transfer studies using the new derivatives are in progress.

Experimental Section

General: All reagents were of analytical grade and used as supplied. Solvents were of the highest grade available. NMR spectra were recorded with a Bruker AC 250, Bruker AMX 500 or Bruker DMX 500 spectrometer. ESI mass spectra were measured with a TSQ 700 Finnigan or Bruker APEX-QIII, 7T spectrometer. A Perkin−Elmer 241 MC polarimeter was used for optical rotation measurements. A Büchi SMP-20 apparatus was used for melting point determination. HPLC was performed using either a Beckman System Gold or Pharmacia Äkta Basic using YMC-Pack ODS, RP-C18 columns for preparative runs (250 × 20 mm, 5 μm, 120 Å) and for analytical samples (250 × 4.6 mm, 5 μm, 120 Å). The optical purity was determined by HPLC analysis of the amino acid dimers obtained with (*S*)-Boc-Ala-OSu.

1,8:4,5-Naphthalenetetracarboxydiimide Derivative 1: *n*-Butylamine (213 μL, 2.15 mmol) was added dropwise under argon to a solution of naphthalene-1,4,5,8-tetracarboxylic dianhydride (**4**; 500 mg, 2.15 mmol) in dry DMF (25 mL). The reaction mixture was heated to 130 °C and stirred for 12 h. After evaporation of the solvent under reduced pressure, the crude material was purified by flash chromatography on silica gel with dichloromethane/acetone (95:5) as eluent. After evaporation of the solvent, the residue was dissolved in dry DMF (10 mL) and treated under argon with (*S*)-*N*-Boc-ornithine (**3**; 470 mg, 2.15 mmol). The reaction mixture was heated to 120 °C and stirred for 18 h. The solvent was evaporated under reduced pressure. Purification of the residue was performed by flash chromatography on silica gel with chloroform/methanol (9:1) as eluent to give **1** (500 mg, 41%) as a pale-green solid. M.p. 252 °C (decomposition). R_F (chloroform/methanol, 9:1) = 0.40. $[\alpha]_D^{25}$ = +37.9 (DMSO, *c* = 0.31). IR (KBr): $\tilde{\nu}$ = 3394 br, 2961 s, 1707 s, 1666 s, 1581 s, 1517 m, 1456 s, 1375 s, 1338 s, 1245 s, 1167 s, 1079 m, 1059 m, 880 w, 771 s, 583 w cm^{-1}. ^{1}H NMR (250 MHz, [D$_6$]DMSO, room. temp.): δ = 0.89−0.95 [t, 3 H, 4-H (*n*Bu)], 1.31 (s, 9 H, *t*Bu), 1.25−1.45 [m, 2 H, 3-H (*n*Bu)], 1.55−1.75 [m, 6 H, β-H, γ-H, 2-H (*n*Bu)], 3.62−3.77 (m, 1 H, α-H), 3.92−4.08 [m, 4 H, δ-H, 1-H (*n*Bu)], 6.41 (br. s, 1 H, NH), 8.60 (s, 4 H, Ar-H) ppm. ^{13}C NMR (62.5 MHz, [D$_6$]DMSO, room. temp.): δ = 14.0, 20.1, 28.2, 28.5, 28.8, 29.9, 53.8, 78.3, 79.6, 126.5, 126.6, 130.0, 154.3, 162.9 ppm. ESI-MS: *m*/*z* (%) = 560.0 (100) [M + Na]$^+$. HRMS (ESI) $C_{28}H_{31}N_3O_8$ (537.6): calcd. 538.2184; found 538.2186 [M + H]$^+$.

Pyromellitdiimide Derivative 2: Pyromellitic dianhydride (**5**; 470 mg, 2.15 mmol) and (*S*)-*N*-Boc-ornithine (**3**; 500 mg, 2.15 mmol) were dissolved in dry DMF (25 mL) under argon. *n*-Butylamine (157 mg, 2.15 mmol, 213 μL) was added dropwise to the suspension with vigorous stirring. The mixture was heated at 130 °C for 20 h. The solvent was evaporated under reduced pressure. Purification was performed by flash chromatography on silica gel with chloroform, followed by a methanol gradient (2% per 250 mL), as the eluent. The white solid obtained after evaporation of the solvents was precipitated from a mixture of hexane and chloroform (1:1). The product was collected and dried to provide the pure amino acid **2** (470 mg, 45%) as a slightly yellow solid. M.p. 230 °C (decomposition). R_F (chloroform/methanol, 4:1) = 0.50. $[\alpha]_D^{25}$ = −19.4 (DMSO, *c* = 0.34). IR (KBr): $\tilde{\nu}$ = 3387 br, 2964 s, 2874 s, 1698 s, 1516 m, 1396 s, 1252 m, 1163 s, 1093 m, 1045 s, 932 m, 895 w, 853 w, 821 w, 800 w, 780 w, 727 s, 609 w, 560 w, 462 w cm^{-1}. ^{1}H NMR (250 MHz, [D$_6$]DMSO, room. temp.): δ = 0.86−0.92 [t, 3 H, 4-H (*n*Bu)], 1.32 (s, 9 H, *t*Bu), 1.37−1.39 [m, 2 H, 3-H (*n*Bu)], 1.53−1.76 [m, 6 H, β-H, γ-H, 2-H (*n*Bu)], 3.58−3.64 [m, 4 H, δ-H, 1-H (*n*Bu)], 3.79−3.83 (m, 1 H, α-H), 7.00 (d, ^3J = 8 Hz, 1 H, NH), 8.17 (s, 2 H, Ph). ^{13}C NMR (62.5 MHz, [D$_6$]DMSO, room. temp.): δ = 13.8, 19.8, 25.3, 28.3, 28.5, 30.2, 53.6, 78.3, 79.8, 117.5, 137.4, 166.7, 170.4, 174.3 ppm. ESI-MS: *m*/*z* (rel. %) = 510.0 (100) [M + Na]$^+$. HRMS (ESI) of $C_{24}H_{28}N_3O_8$ (486.5): calcd. 510.1847; found 510.1844 [M + Na]$^+$.

2-Chlorotrityl Resin-Bound (*S*)-*N*-*tert*-Butoxycarbonyl-β-*N*-(9-fluorenylmethoxycarbonyl)aminoalanine (9): 2-Chlorotrityl resin (3.00 g,

940 μmol Cl/g) was weighed in a fritted reaction chamber. The resin was swelled for 20 min in dichloromethane. (*S*)-*N*-*tert*-Butoxycarbonyl-β-*N*-(9-fluorenylmethoxycarbonyl)aminoalanine (**8**; 1.50 g, 3.50 mmol) was suspended in dry dichloromethane (30 mL) and then *N*,*N*-diisopropylethylamine (400 μL, 2.30 mmol) was added. The solution was stirred until the solid had dissolved. This solution was added to the swelled resin and the mixture was shaken for 5 min. Another portion of *N*,*N*-diisopropylethylamine (800 μL, 4.60 mmol) was then added and the mixture shaken for another 2 h. Methanol (3 mL) was then added and mixed for 20 min. The resin was washed two times with dichloromethane and three times with DMF. After drying for 48 h under vacuum, the modified resin **9** was collected. The loading of the resin was calculated by weight difference to be 587 μmol **8**/g resin **9**.

(*S*)-*N*-*tert*-Butoxycarbonyl-β-(aminoalanyl) 1-Pyrenylacetoamide (6): The amino acid-loaded resin **9** (501 mg, 587 μmol/g resin) was placed in a 10-mL fritted syringe. The resin was swelled for 2 h in dry DMF (9 mL). After removing the solvent, 20% piperidine in DMF (5 mL) was added and reacted with the resin for 5 min. Another portion of 20% piperidine in DMF (5 mL) was then added and the reaction proceeded for another 20 min. The resin was then washed four times with DMF (8 mL) over 2 min. Pyreneacetic acid (193 mg, 741 μmol), TBTU (238 mg, 741 μmol), and 1-hydroxy-1*H*-benzotriazole (100 mg, 741 μmol) were dissolved in *N*-methyl-2-pyrrolidone (7 mL) and then added to the resin. *N*,*N*-Diisopropylethylamine (151 μL, 1.76 mmol) was added to initiate coupling. The syringe was shielded from light and the reaction mixture was shaken for 3 h. The resin was then washed four times, for 2 min each time, with DMF (10 mL). To complete the reaction, the resin was reacted a second time with 1-pyreneacetic acid, TBTU, HOBT, and DIPEA, followed by two washes with DMF. The solid was then washed three times with dichloromethane (10 mL). The final product was obtained after cleavage from the resin using a mixture of acetic acid, trifluoroacetic acid, and dichloromethane (3:1:6). After evaporation under reduced pressure and drying under HV, product **6** was collected (130 mg, 8% from **8**). M.p. 137 °C. R_F (chloroform/methanol/water/acetic acid, 70:30:3:0.3) = 0.60. $[\alpha]_D^{25} = -20.0$ (acetone, $c = 0.31$). IR (KBr): $\tilde{\nu}$ = 3335 br, 3042 m, 2976 s, 2929 s, 1927 w, 1712 br, 1652 s, 1525 s, 1433 m, 1391 m, 1366 s, 1345 m, 1249 s, 1162 s, 1061 m, 1026 w, 966 w, 845 s, 779 w, 756 w, 710 m, 598 w cm^{-1}. ^1H NMR (250 MHz, [D$_6$]DMSO, room. temp.): δ = 1.35 (s, 9 H, *t*Bu), 3.45−3.60 (m, 2 H, β-H), 4.06 (m, 1 H, α-H), 4.20 (s, 2 H, CH_2-Ar), 6.96 (d, ^3J = 8 Hz, 1 H, NH), 7.96−8.35 (m, 9 H, Ar-H) ppm. ^{13}C NMR (62.5 MHz, [D$_6$]DMSO, room. temp.): δ = 28.5, 41.7, 53.8, 78.6, 80.3, 124.3, 124.4, 124.5, 125.2, 125.3, 125.4, 126.5, 127.2, 127.6, 127.7, 129.0, 130.1, 130.7, 131.0, 131.2, 131.7, 155.7, 163.4, 171.1 ppm. ESI-MS: *m*/*z* (%) = 347.0 (82) $[M - Boc + 2H]^+$, 390.8 (44) $[M - tBu + 2H]^+$, 469.0 (35) $[M + Na]^+$, 892.8 (100) $[2M + H]^+$, 914.9 (51) $[2M + Na]^+$, 1338.5 (7) $[3M + H]^+$, 1360.7 (10) $[3M + Na]^+$. HRMS (ESI) of $C_{26}H_{26}N_2O_5$ (446.5): calcd. 447.1915; found 447.1912 $[M + H]^+$.

(*S*)-β-(2-Amino-6-chloro-7-carbapurin-9-yl)-*N*-(*tert*-butoxycarbonyl)alanine (17): 2-Amino-7-carba-6-chloropurine (**15**, 1.80 g, 10.7 mmol) was dissolved in absolute DMSO (15 mL) and treated with *N*-Boc-L-serine lactone (**14**, 2.00 g, 10.7 mmol) in a darkened apparatus. After adding DBU (1.63 g, 10.7 mmol, 1.60 mL), the reaction mixture was stirred at room temperature for 2 h. After removal of the solvent, purification of the product was achieved by flash chromatography on silica gel with ethyl acetate/methanol/acetic acid (80:20:5) as the eluent to give **17** (584 mg, 15%) as a slightly yellow solid. R_f = 0.45 (chloroform/methanol/water/acetic acid, 70:30:3:0.35). ^1H NMR (250 MHz, [D$_6$]DMSO, room. temp.): δ = 0.99 (br. s, 1.5 H, *t*Bu rotamer), 1.25 (br. s, 7.5 H, *t*Bu), 3.99 (m, 1 H, β-H), 4.16 (m, 1 H, α-H), 4.55 (dd, ^3J = 14, ^4J = 4 Hz, 1 H, β-H), 6.20 (d, ^3J = 4 Hz, 1 H, 7-H), 6.60−6.69 (m, 3 H, BocN*H*, NH_2), 7.04 (d, ^3J = 4 Hz, 1 H, 8-H) ppm. ^{13}C NMR (62.5 MHz, [D$_6$]DMSO, room. temp.): δ = 28.3 [$C_{CH(Boc)}$], 45.7 ($C_{H\beta}$), 54.5 ($C_{H\alpha}$), 78.1 [$C_{C(Boc)}$], 98.1 (C-7), 108.0 (C-5), 127.4 (C-8), 151.0 (C-4), 153.9 (C-6), 155.1 (C_{BocCO}), 159.4 (C-2), 172.3 (C_{COOH}) ppm. ESI-MS: *m*/*z* (%) = 356.2 (100) $[M + H]^+$. HRMS (EI) of $C_{14}H_{18}ClN_5O_4$ (355.8): calcd. 355.1047; found 355.1047 $[M^+]$.

(S)-*N*-*tert*-Butoxycarbonyl-β-(7-carbaguanin-9-yl)alanine (12): (*S*)-*N*-*tert*-Butoxycarbonyl-β-(2-amino-7-carba-6-chloro-9-purinyl)-alanine (**17**; 100 mg, 281 μmol) was dissolved in 1 N hydrochloric acid (10 mL) and heated for 18 h at 80 °C. After removal of the solvent, the brown residue was dissolved in a mixture of sodium hydroxide, water, and dioxane (1:1:2) and then cooled to 0 °C. Di-*tert*-butylhydrogencarbonate was added and the reaction mixture was stirred for 45 min at 0 °C. The dark-brown solution was warmed slowly to room temperature and then stirred for another 72 h. After evaporation of the solvent, the crude product was purified by flash chromatography on silica gel with ethyl acetate/methanol/acetic acid (80:20:5) as eluent to give **12** (37 mg, 39%, *ee* = 99.4%) as a white solid. R_f = 0.53 (chloroform/methanol/water/acetic acid, 70:30:3:0.35). $[\alpha]_D^{25} = +55.2$ (MeOH, $c = 0.19$). M.p. 234 °C. IR (KBr): $\tilde{\nu}$ = 3368 br, 2925 s, 2846 s, 2362 m, 1654 s, 1560 s, 1543 s, 1528 s, 1508 s, 1458 m, 1396 s, 1253 m, 1165 m, 1051 m, 854 w, 785 w, 727 w, 681 w cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO, room. temp.): δ = 1.07 (br. s, 1.5 H, *t*Bu rotamer), 1.27 (br. s, 7.5 H, *t*Bu), 3.85 (m, 1 H, α-H or β-H), 4.02 (m, 1 H, α-H or β-H), 4.45 (dd, ^3J = 13, ^4J = 3 Hz, 1 H, β-H), 6.10 (d, ^3J = 3 Hz, 1 H, 7-H), 6.25 (d, ^3J = 8 Hz, 1 H, BocN*H*), 6.52 (br. s, NH_2), 6.63 (d, ^3J = 3 Hz, 1 H, 8-H), 10.89 (br. s, 1 H, 1-H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO, room. temp.): δ = 28.0 [$C_{CH(Boc)}$], 28.4 [$C_{CH(Boc)}$], 46.2 ($C_{H\beta}$), 55.9 ($C_{H\alpha}$), 77.7 [$C_{C(Boc)}$], 99.9 (C-5), 100.6 (C-7), 121.0 (C-8), 150.8 (C-4), 152.7 (C-2), 155.0 (C_{BocCO}), 159.2 (C-6), 173.7 (C_{COOH}) ppm. ESI-MS: *m*/*z* (%) = 338.1 (100) $[M + H]^+$.

(S)-*N*-*tert*-Butoxycarbonyl-β-(6-chloro-7-carbapurin-9-yl)alanine (18): *N*-Boc-L-serine lactone (**14**, 1.00 g, 5.70 mmol) was added to a solution of 6-chloro-7-carbapurine (1.75 g, 11.4 mmol) in absolute DMSO (15 mL). After adding DBU (868 mg, 5.70 mmol, 852 μL), the reaction mixture was stirred for 1 h at room temperature. After evaporation of the solvent, purification of the crude product was performed by flash chromatography on silica gel (ethyl acetate/methanol/acetic acid, 80:20:5) to give **18** (1.03 g, 53%) as a slightly yellow solid. R_f = 0.56 (chloroform/methanol/water/acetic acid, 70:30:3:0.35). ^1H NMR (250 MHz, [D$_6$]DMSO, room. temp.): δ = 0.94 (br. s, 1.5 H, *t*Bu rotamer), 1.17 (br. s, 7.5 H, *t*Bu), 4.17−4.35 (m, 2 H, α-H, β-H), 4.75 (m, 1 H, β-H), 6.54 (d, ^3J = 4 Hz, 1 H, 7-H), 6.58 (m, 0.2 H, BocN*H*), 6.67 (d, ^3J = 6 Hz, 0.8 H, BocN*H*), 7.60 (d, ^3J = 4 Hz, 1 H, 8-H), 8.56 (s, 1 H, 2-H) ppm. ^{13}C NMR (62.5 MHz, [D$_6$]DMSO, room. temp.): δ = 26.5 [$C_{CH(Boc)}$], 28.4 [$C_{CH(Boc)}$], 46.2 ($C_{H\beta}$), 54.4 ($C_{H\alpha}$), 78.2 [$C_{C(Boc)}$], 98.3 (C-7), 117.0 (C-5), 128.4 (C-8), 150.2 (C-2), 150.6 (C-4, C-6), 151.2 (C-4, C-6), 155.0 (C_{BocCO}), 172.2 (C_{COOH}) ppm. ESI-MS: *m*/*z* (%): 341.1 (100) $[M + H]^+$. HRMS (ESI) $[M + H]^+$ ($C_{14}H_{17}ClN_4O_4$): calcd. 341.1011; found 341.1009.

(*S*)-*N*-*tert*-Butoxycarbonyl-β-(6-chloro-7-carbapurin-9-yl)alanine Benzyl Ester (19): A solution of (*S*)-*N*-*tert*-butoxycarbonyl-β-(-6-chloro-7-carbapurin-9-yl)alanine (**18**, 990 mg, 2.91 mmol) in anhydrous DMF (5 mL) was diluted with benzyl alcohol (5 mL). After

cooling to 0 °C, DMAP (354 mg, 2.91 mmol) and EDCI·HCl (612 mg, 3.20 mmol) were added and the reaction mixture was warmed to room temperature and stirred for 18 h. After evaporation of the solvents, purification of the residue was achieved by flash chromatography on silica gel (ethyl acetate/hexane, 1:1) to give **19** (1.14 g, 91%) as a white solid. R_f = 0.68 (ethyl acetate/hexane, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$, room. temp.): δ = 1.11 (br. s, 1.5 H, *t*Bu rotamer), 1.26 (br. s, 7.5 H, *t*Bu), 4.45−4.58 (m, 2 H, α-H, β-H), 4.74 (dd, ^3J = 13, ^4J = 5 Hz, 1 H, β-H), 5.08 (dt, 2 H, PhCH_2), 6.60 (d, ^3J = 4 Hz, 1 H, 7-H), 7.23−7.38 (m, 5 H, Ph), 7.46 (d, ^3J = 8 Hz, 1 H, BocN*H*), 7.67 (d, ^3J = 4 Hz, 1 H, 8-H), 8.61 (s, 1 H, 2-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, room. temp.): δ = 27.7 [$C_{CH(Boc)}$], 28.3 [$C_{CH(Boc)}$], 45.2 ($C_{H\beta}$), 53.7 ($C_{H\alpha}$), 66.7 (C_{CHPh}), 79.0 [$C_{C(Boc)}$], 98.9 (C-7), 117.2 (C-5), 128.2 (C_{Phenyl}, C-8), 128.4 (C_{Phenyl}, C-8), 128.7 (C_{Phenyl}, C-8), 132.3 (C_{Phenyl}, C-8), 135.8 ($C_{Phenyl\ C\text{-}1}$), 150.6 (C-2), 150.9 (C-4, C-6), 151.3 (C-4, C-6), 155.5 (C_{BocCO}), 170.2 (C_{COOBzl}) ppm. ESI-MS: *m*/*z* (%) = 431.2 (100) [M + H]$^+$. HRMS (ESI) $C_{21}H_{23}ClN_4O_4$ (430.9): calcd. 431.1481; found 431.1478 [M + H]$^+$.

(*S*)-β-(6-Azido-7-carbapurin-9-yl)-*N*-(*tert*-butoxycarbonyl)alanine Benzyl Ester (20) and (*S*)-*N*-*tert*-Butoxycarbonyl-β-(tetrazolo[6,1-*i*]purin-9-yl)alanine Benzyl Ester (21): Sodium azide (2.46 g, 38.0 mmol) was added to a solution of (*S*)-*N*-*tert*-butoxycarbonyl-β-(6-chloro-7-carbapurin-9-yl)alanine benzyl ester (1.09 g, 2.53 mmol) in anhydrous DMF (25 mL). The reaction mixture was stirred at 60 °C for 18 h. The solvent was evaporated and the crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane, 2:3) to give a mixture of **20** and **21** (251 mg, 23%; ratio 3:97) as a white solid. R_f = 0.47 (ethyl acetate/hexane, 2:3). ^{1}H NMR (250 MHz, $CDCl_3$, room. temp.): δ = 1.29 (br. s, 9 H, *t*Bu), 4.28−4.37 (m, 1 H, β-H), 4.55−4.84 (m, 2 H, α-H, β-H), 4.94−5.10 (m, 2 H, PhCH_2), 5.25 (d, ^3J = 5 Hz, 1 H, BocN*H*), 6.40 (d, ^3J = 3 Hz, 0.03 H, 7-H, azide), 6.91 (d, ^3J = 3 Hz, 0.97 H, 7-H, tetrazole), 7.02−7.30 (m, 6 H, 8-H, Ph), 8.46 (s, 0.03 H, 2-H, azide), 9.02 (s, 0.97 H, 2-H, tetrazole) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$, room. temp.): δ = 28.7 [$C_{CH(Boc)}$], 47.4 ($C_{H\beta}$), 54.7 ($C_{H\alpha}$) 60.9, 65.8, 67.9, 78.0 [$C_{C(Boc)}$], 102.7 (C-7), 103.8 (C-5), 128.1, 128.7, 129.0, 129.3, 131.6, 134.8, 147.1, 171.2 (C_{COOBzl}) ppm. ESI-MS: *m*/*z* (%) = 438.2 (100) [M + H]$^+$, 460.2 (21) [M + Na]$^+$. HRMS (ESI) of $C_{21}H_{23}N_7O_4$ (437.5): calcd. 438.1884; found 438.1883 [M + H]$^+$.

(*S*)-*N*-*tert*-Butoxycarbonyl-β-(7-carbaadenin-9-yl)alanine (13): A mixture of (*S*)-*N*-*tert*-butoxycarbonyl-β-(6-chloro-7-carbapurin-9-yl)alanine benzyl ester (**20**) and (*S*)-*N*-*tert*-butoxycarbonyl-β-(tetrazolo[6,1-*i*]purin-9-yl)alanine benzyl ester (**21**) (250 mg, 571 μmol) were dissolved in a mixture of dioxane (10 mL), water (6.25 mL) and glacial acetic acid (500 μL). Palladium on charcoal (60.0 mg, 5% Pd containing 50.5% water) was added and the mixture was kept for 24 h under an atmosphere of hydrogen gas. The reaction product was separated from the charcoal by filtration and subsequent washing with methanol. The solvent was evaporated and the product isolated by flash chromatography on silica gel (ethyl acetate/methanol/acetic acid, 80:20:5) to give **13** (61 mg, 33%; *ee* = 88.5%) as a white solid. R_f = 0.26 (chloroform/methanol/water/acetic acid, 70:30:3:0.35). $[\alpha]_D^{25}$ = +43.4 (MeOH, *c* = 0.14). M.p. 164−168 °C (dec.). IR (KBr): $\tilde{\nu}$ = 3676 s, 3448 s, 2925 m, 2362 m, 2545 m, 1943 w, 1918 w, 1717 s, 2654 s, 1560 s, 1522 s, 1508 s, 1490 s, 1419 s, 1363 s, 1340 m, 1168 m, 1052 m, 1025 m, 669 m, 518 w, 472 w cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO, room. temp.): δ = 1.01 (br. s, 1.5 H, *t*Bu rotamer), 1.25 (br. s, 7.5 H, *t*Bu), 3.88−4.12 (m, 2 H, α-H, β-H), 4.66 (dd, ^3J = 13, ^4J = 3 Hz, 1 H, β-H), 5.93 (m, 1 H, BocN*H*), 6.40 (d, ^3J = 3 Hz, 1 H, 7-H), 6.78 (br. s, 2 H, NH_2), 7.03 (s, ^3J = 3 Hz, 1 H, 8-H), 7.98 (s, 1 H, 2-H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO, room. temp.): δ = 28.5 [$C_{CH(Boc)}$], 48.9 ($C_{H\beta}$), 56.3 ($C_{H\alpha}$), 77.6 [$C_{C(Boc)}$], 98.1 (C-7), 102.6 (C-1), 125.1 (C-8), 150.5 (C-4), 151.5 (C-2), 154.9 (C_{BocCO}), 157.6 (C-6), 175.1 (C_{COOH}) ppm. ESI-MS: *m*/*z* (%) = 322.1 (100) [M + H]$^+$. HRMS (ESI) of $C_{14}H_{19}N_5O_4$ (321.3): calcd. 322.1510; found 322.1507 [M + H]$^+$.

(*S*)-β-(2-Aminopurin-9-yl)-*N*-(*tert*-butoxycarbonyl)alanine (22): (*S*)-*N*-*tert*-Butoxycarbonyl-β-(2-amino-6-chloropurin-9-yl)alanine (**23**, 830 mg, 2.33 mmol) was dissolved in a mixture of methanol (100 mL) and glacial acetic acid (3 mL). Palladium on charcoal (111 mg, 5% Pd on charcoal containing 50.5% water) was added, the mixture was saturated with hydrogen gas, and then left to react for 38 h. The charcoal was separated from the reaction product by filtration and then washed with methanol. The solvent was evaporated and the product isolated by flash chromatography on silica gel (ethyl acetate/methanol/acetic acid, 80:20:5) to give **22** (437 mg, 58%; *ee* = 98.2%) as a white solid. R_f = 0.60 (2-propanol/water/acetic acid, 5:2:1, saturated with sodium chloride). $[\alpha]_D^{25}$ = −33.9 (MeOH, *c* = 0.2). M.p. 211 °C (decomp.). IR (KBr): $\tilde{\nu}$ = 3350 br, 3216 s, 2879 m, 1618 s, 1583 s, 1524 s, 1477 s, 1429 s, 1394 s, 1295 m, 1254 m, 1165 s, 1055 m, 1025 w, 965 w, 901 w, 854 w, 796 m, 638 m cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO, room. temp.): δ = 0.97 (br. s, 1.5 H, *t*Bu rotamer), 1.24 (br. s, 7.5 H, *t*Bu), 4.00−4.23 (m, 2 H, α-H, β-H), 4.45−4.55 (m, 1 H, β-H), 6.49 (br. s, 2 H, NH_2), 6.68 (d, ^3J = 7 Hz, 1 H, BocN*H*), 7.88 (s, 1 H, 8-H), 8.51 (s, 1 H, 6-H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO, room. temp.): δ = 28.2 [$C_{CH(Boc)}$], 44.3 ($C_{H\beta}$), 54.3 ($C_{H\alpha}$), 78.1 [$C_{C(Boc)}$], 127.0 (C-5), 143.3 (C-6), 148.8 (C-8), 153.4 (C-4), 155.2 (C_{BocCO}), 160.6 (C-2), 172.2 (C_{COOH}) ppm. ESI-MS: *m*/*z* (%) = 323.1 (100) [M + H]$^+$. HRMS (ESI) of $C_{14}H_{18}N_6O_4$ (334.3): calcd. 322.1462; found 322.1460 [M + H]$^+$.

(*S*)-β-(2-Amino-7-carbapurin-9-yl)-*N*-(*tert*-butoxycarbonyl)alanine (24): (*S*)-*N*-*tert*-Butoxycarbonyl-β-(2-amino-6-chloro-7-carbapurin-9-yl)alanine (**17**; 58.0 mg, 163 μmol) was dissolved in a mixture of methanol (20 mL) and glacial acetic acid (600 μL). Palladium on charcoal (8.0 mg, 5% Pd on charcoal containing 50.5% water) was added and then the mixture was saturated with hydrogen gas and left to react for 90 min. The charcoal was separated from the reaction product by filtration and subsequent washing with methanol. The solvent was evaporated and the product was isolated by flash chromatography on silica gel (ethyl acetate/methanol/acetic acid, 80:20:5) to give **24** (21 mg, 40%; *ee* = 85.1%) as a slightly yellow solid. R_f = 0.56 (chloroform/methanol/water/acetic acid, 70:30:3:0.35). ^{1}H NMR (400 MHz, [D$_6$]DMSO, room. temp.): δ = 0.96 (br. s, 1.5 H, *t*Bu rotamer), 1.24 (br. s, 7.5 H, *t*Bu), 3.88−4.12 (m, 2 H, α-H, β-H), 4.58 (dd, ^3J = 13, ^4J = 4 Hz, 1 H, β-H), 6.07 (br. s, 2 H, NH_2), 6.14 (d, ^3J = 7 Hz, 1 H, BocN*H*), 6.18 (d, ^3J = 3 Hz, 1 H, 7-H), 6.99 (d, ^3J = 3 Hz, 1 H, 8-H), 8.38 (s, 1 H, 6-H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO, room. temp.): δ = 27.5 [$C_{CH(Boc)}$], 28.3 [$C_{CH(Boc)}$], 45.8 ($C_{H\beta}$), 55.6 ($C_{H\alpha}$), 77.5 [$C_{C(Boc)}$], 98.5 (C-7), 111.2 (C-5), 126.6 (C-8), 150.0 (C-6), 153.0 (C-4), 154.9 (C_{BocCO}), 159.9 (C-2), 172.8 (C_{COOH}) ppm. ESI-MS: *m*/*z* (%) = 322.2 (100) [M + H]$^+$. HRMS (ESI) of $C_{14}H_{19}N_5O_4$ (321.3): calcd. 322.1510; found 322.1506 [M + H]$^+$.

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