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A second-generation copper(II)-mediated metallo-DNA-base pair

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Abstract

Metal-dependent pairing of nucleobases represents an alternative DNA base pairing scheme. Our first-generation copper(II)-mediated pyridine-2,6-dicarboxylate (**Dipic**) and pyridine (**Py**) metallo-base pair has a stability comparable to the natural base pairs dA:dT and dC:dG but does not have the selectivity of the Watson Crick base pairs. In order to increase the selectivity of base pair formation, a second-generation metallo-base pair was generated consisting of a pyridine-2,6-dicarboxamide (**Dipam**) and a pyridine (**Py**) nucleobase. This new metallo-base pair is more stable than the natural base pairs dA:dT and dC:dG and highly selective against mispairing. In addition, incorporation of multiple metallo-base pairs into DNA results in the formation of stable duplexes demonstrating that hydrogen bonding base pairs can efficiently be replaced by metal-dependent base pairs at multiple sites in DNA.

Keywords: Nucleobases; Alternative binding schemes; Metallo-DNA-base pairs; Copper(II); Tridentate pyridine ligands

1. Introduction

Watson–Crick hydrogen bonding of adenine with thymine (dA:dT base pair) and guanine with cytosine (dG:dC base pair) is essential for the stability and specificity of duplex formation. Additional, stable and selective base pairs would increase the

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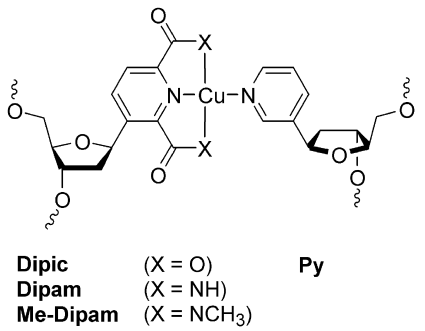


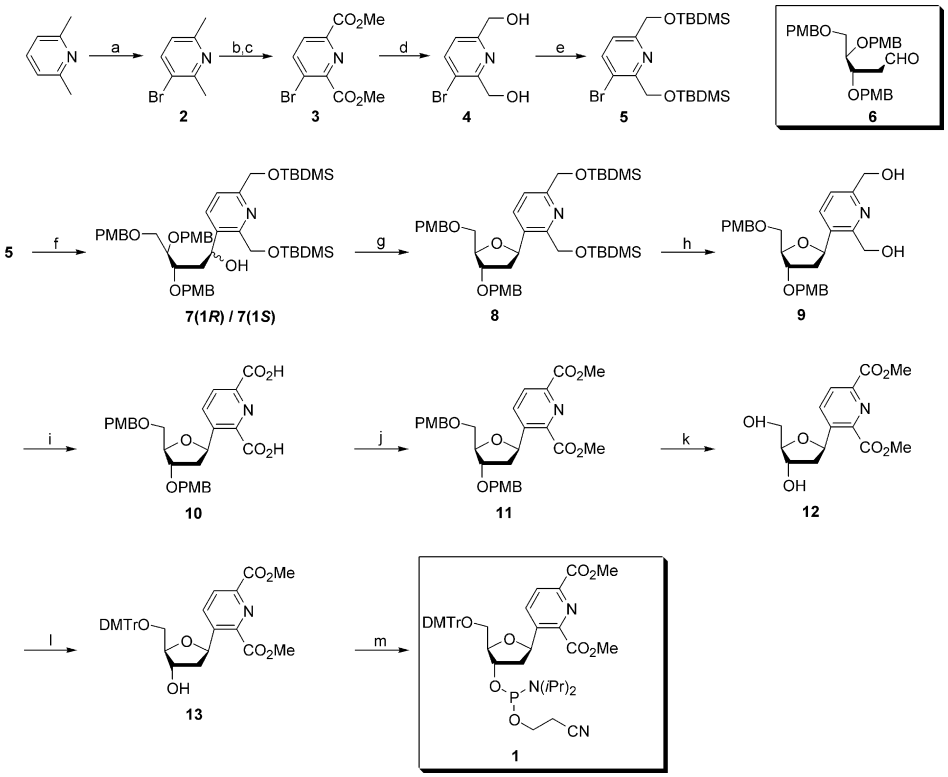
Fig. 1. Copper(II)-mediated metallo-base pairing between the tridentate nucleobases **Dipic**, **Dipam** or **Me-Dipam** and a monodentate pyridine (**Py**) nucleobase.

capacity of DNA for information storage and might allow the generation of DNAs with novel functions [1,2]. Efforts to develop such a third unnatural base pair have focused on two different strategies thus far: the design of bases with altered hydrogen bonding patterns [3] or hydrophobic packing interactions [4,5]. We and others recently reported another strategy in which hydrogen bonding interactions are replaced by metal-dependent pairing of two nucleobases [6,7]. Our first generation metallo-base pair involved a pyridine-2,6-dicarboxylate nucleobase (**Dipic**) as a planar tridentate ligand, and a pyridine nucleobase (**Py**) as the complementary single donor ligand (Fig. 1) [6]. When these bases are introduced into the middle of a 15-nucleotide duplex, the duplex displays almost comparable melting temperatures (T_m) in the presence of equimolar Cu^{2+} to a duplex with a dA:dT pair. However, this metallo-base pair has only modest pairing selectivity. Relatively stable mispairs are formed when paired with natural bases, especially with the purine nucleobases dA and dG. In order to store genetic information, an unnatural base pair should not only be highly stable but also highly selective against mispairing with the natural nucleobases. Herein we report the design and synthesis of a new copper(II)-mediated metallo-base pair consisting of a tridentate pyridine-2,6-dicarboxamide ligand (**Dipam**) and a monodentate pyridine ligand (**Py**) (Fig. 1). This second-generation metallo-base pair shows high specificity against mispairing with the natural nucleobases while maintaining a high pairing stability.

2. Experimental

2.1. General

All reactions were carried out in oven-dried glassware under inert atmosphere with commercial dehydrated solvents (Sigma–Aldrich) unless otherwise stated. *Aldehydo*-D-ribose **6** was prepared according to known literature procedures [8]. The synthesis of the **Py** nucleobase followed the same route as shown in Scheme 1 and is a modification of the first reported synthesis for this nucleobase (for analytical data see



Scheme 1. Synthetic route for phosphoramidite **1**[a]. [a]Conditions: (a) Br_2, oleum (27–30% SO_3), reflux (57%). (b) $KMnO_4$, H_2O, 90 °C (60%). (c) $SOCl_2$, reflux, then MeOH (78%). (d) $NaBH_4$, EtOH, reflux (77%). (e) TBDMS triflate, 2,6-lutidine, CH_2Cl_2, rt (98%). (f) *n*-BuLi, ether, −78 °C, then *aldehydo*-D-ribose **6**, THF (35% **7**(**1R**)), (36% **7**(**1S**)). (g) $MeSO_2Cl$, NEt_3, pyridine, 0 °C, then rt (60%). (h) TBAF, THF, rt (98%). (i) TEMPO, NaOCl, $NaClO_2$, CH_3CN, phosphate buffer (pH 6.7), 35 °C (84%). (j) trimethylsilyl diazomethane, benzene, MeOH, rt (59%). (k) TFA, CH_2Cl_2, rt (98%). (l) DMTr-Cl, NEt_3, py, rt (53%). (m) $(i\text{-Pr})_2NP(Cl)CH_2CH_2CN$, $(i\text{-Pr})_2NEt$, CH_2Cl_2, rt (88%).

[8]). Oligonucleotides were synthesized using an Expedite nucleic acid synthesizer (PerSeptive Biosystems). DNA synthesis reagents were purchased from Glen Research, Sterling, VA. All other reagents were purchased from Sigma–Aldrich.

2.2. Synthesis

2.2.1. 3-Bromo-2,6-dimethyl-pyridine (**2**)

2,6-Lutidine (50 mL, 0.43 mol) was slowly added to oleum (27–30% SO_3, 250 mL) at 0 °C, followed by the addition of bromine (9.0 mL, 0.18 mol). The reaction mixture was stirred at 150 °C for 18 h. After cooling to RT the reaction mixture was poured into ice-water (1 L), neutralized by careful addition of 5 M NaOH (2 L) and extracted with ethyl acetate (5×600 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated. Vacuum destillation [bp: 95 °C (15 mbar)] afforded **2**

(37.9 g, 57%) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.62 (1H, d, $J = 8.1$ Hz), 6.82 (1H, d, $J = 8.1$ Hz), 2.59 (3H, s), 2.44 (3H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 156.5, 156.3, 139.8, 122.1, 118.2, 24.9, 23.9.

*2.2.2. 3-Bromo-pyridine-2,6-dicarboxylic acid dimethyl ester (**3**)*

To a solution of compound **2** (37.9 g, 0.20 mol) in water (500 mL) was added $KMnO_4$ (171.0 g, 1.08 mol) in ten portions. The first five portions were added over 5 h at 70 °C and the second five portions over 5 h at 90 °C. After complete addition stirring was continued at 90 °C for 12 h. The reaction mixture was filtered hot and the residue was washed with hot water (4×100 mL). The filtrate was concentrated (ca. 400 mL) and conc. HCl (65 mL) was added. Heating to 100 °C redissolved the precipitated white solid, and the filtrate was stored at 4 °C overnight. The resulting white solid was filtered, washed with ice-cold water (60 mL) and air-dried to give 5-bromo-pyridine-2,5-dicarboxylic acid (29.8 g, 60%); (^{1}H-NMR (250 MHz, d_6-DMSO) δ 8.18 (1 H, d, $J = 8.4$ Hz), 7.82 (1H, d, $J = 8.4$ Hz)). The dicarboxylic acid (28.8 g, 0.12 mol) was dissolved in thionyl chloride (100 mL) and heated to reflux for 4 h. After cooling to RT thionyl chloride was removed *in vacuo* and MeOH (200 mL) was added. The resulting solution was heated to reflux for 1 h, concentrated and dried under high vacuum to give compound **3** (25.0 g, 78%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.13 (1H, d, $J = 8.4$ Hz), 8.06 (1H, d, $J = 8.3$ Hz), 4.00 (3H, s), 3.99 (3H, s). ESMS calcd for $C_9H_8BrNO_4$ (MH^+): 274.0; found: 274/276.

*2.2.3. (5-Bromo-6-hydroxymethyl-pyridin-2-yl)-methanol (**4**)*

To a suspension of compound **3** (25.0 g, 91 mmol) in EtOH (225 mL) at 0 °C was added $NaBH_4$ (16.2, 0.43 mol) in portions over 0.5 h. The reaction mixture was stirred for 3 h at RT and then heated to reflux for 16 h. After cooling to RT the reaction mixture was poured into saturated aqueous $NaHCO_3$ (500 mL) and extracted with CH_2Cl_2 (5×200 mL). The combined organic extracts were dried over $MgSO_4$. Chromatography over silica (CH_2Cl_2:MeOH; 10:1) afforded compound **4** (15.3 g, 77%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.81 (1H, d, $J = 8.1$ Hz), 7.18 (1H, d, $J = 8.1$ Hz), 4.73 (2H, s), 4.71 (2H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 157.6, 155.6, 140.8, 120.6, 116.9, 64.5, 63.2. HRMS (MALDI) calcd for $C_7H_8BrNO_2$ (MH+): 217.9817; found: 217.9816.

*2.2.4. 3-Bromo-2,6-bis-(tert-butyl-dimethyl-silanyloxymethyl)-pyridine (**5**)*

To a solution of compound **4** (15.3 g, 70.2 mmol) and 2,6-lutidine (42 mL, 0.36 mol) in CH_2Cl_2 (150 mL) at 0 °C was added TBDMS trifluoromethanesulfonate (43 mL, 0.19 mol) over 0.5 h. Stirring was continued at 0 °C for 1 h, followed by 2 h at RT. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (200 mL) and extracted with CH_2Cl_2 (3×150 mL). The combined organic extracts were washed with brine (150 mL), dried over $MgSO_4$ and concentrated. Chromatography over silica (hexanes:ethyl acetate; 20:1) afforded compound **5** (30.7 g, 98%) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.80 (1H, d, $J = 8.4$ Hz), 7.29 (1H, d, $J = 8.1$ Hz), 4.85 (2H, s), 4.76 (2H, s), 0.94 (9H, s), 0.89 (9H, s), 0.09 (6H, s), 0.08

(6H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 159.8, 156.1, 140.9, 120.3, 118.1, 66.6, 65.6, 26.0, 25.9, 18.5, 18.3, −5.1, −5.4. ESMS calcd for $C_{19}H_{36}BrNO_2Si_2$ (MH^+): 446.2; found: 446/448.

*2.2.5. 1S-[2,6-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-pyridin-3-yl]-3S, 4R,5-tris-(4-methoxy-benzyloxy)-pentan-1-ol (**71S**) and 1R-[2,6-bis-(tert-butyl-dimethyl-silanyloxymethyl)-pyridin-3-yl]-3S,4R,5-tris-(4-methoxy-benzyloxy)-pentan-1-ol (**71R**)*

To a solution of compound **5** (5.5 g, 12.3 mmol) in ether (35 mL) at −78 °C was added dropwise a solution of *n*-BuLi in hexanes (1.6 M, 8.5 mL, 13.6 mmol) over 0.5 h. After stirring for 1 h at −78 °C a solution of aldehyde **6** [8] (6.7 g, 13.5 mmol) in THF (35 mL) was added. After 0.5 h at −78 °C the cooling bath was removed, and stirring was continued at RT for 2 h. The reaction mixture was poured into ice-cold saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3×150 mL). The combined organic extracts were washed with brine (150 mL), dried over $MgSO_4$ and concentrated. Separation of diastereomers was achieved by chromatography over silica (hexanes:ethyl acetate; 8:1, then 5:1). Compound **71R** (3.67 g, 35%) eluted first, followed by compound **71S** (3.81 g, 36%). Both compounds were obtained as colorless oils. **71R**: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.69 (1H, d, $J = 8.1$ Hz), 7.39 (1H, d, $J = 8.1$ Hz), 7.24 (6H, m), 6.84 (6H, m), 5.22 (1H, m), 4.95 (1H, d, $J = 11.7$ Hz), 4.78 (2H, d, $J = 4.0$ Hz), 4.73 (2H, d, $J = 11.7$ Hz), 4.62 (3H, m), 4.49 (1H, d, $J = 11.4$ Hz), 4.44 (2H, d, $J = 3.3$ Hz), 4.00 (1H, m), 3.79 (9H, m), 3.66 (1H, d, $J = 3.7$ Hz), 3.62 (2H, d, $J = 5.1$ Hz), 2.00 (2H, m), 0.95 (9H, s), 0.86 (9H, s), 0.06 (8H, s), 0.04 (4H, s). HRMS (MALDI) calcd for $C_{48}H_{71}NO_9Si_2$ (MNa^+): 884.4565; found: 884.4597. **71S**: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.80 (1H, d, $J = 7.7$ Hz), 7.42 (1H, d, $J = 7.7$ Hz), 7.23 (6H, m), 6.85 (6H, m), 5.21 (1H, m), 4.80 (4H, m), 4.62 (3H, m), 4.42 (2H, m), 4.30 (1H, m), 3.90 (1H, m), 3.81 (9H, s), 3.62 (3H, m), 2.05 (2H, m), 0.96 (9H, s), 0.87 (9H, s), 0.12 (8H, s), 0.06 (4H, s). HRMS (MALDI) calcd for $C_{48}H_{71}NO_9Si_2$ (MH^+): 862.4746; found: 862.4761.

*2.2.6. 2,6-Bis-(tert-butyl-dimethyl-silanyloxymethyl)-3-[4S-(4-methoxy- benzyloxy)-5R-(4-methoxy-benzyloxy-methyl)-tetrahydro-furan-2R-yl]- pyridine (**8**)*

To a solution of compound **71S** (2.96 g, 3.43 mmol) in pyridine (180 mL) at 0 °C was added triethylamine (4.8 mL, 34.3 mmol). A solution of methanesulfonyl chloride (1.06 mL, 13.7 mmol) in pyridine (15 mL) was slowly added over 1 h. After 1 h at 0 °C stirring was continued at RT for 3 h. The reaction mixture was quenched with water (10 mL) and concentrated. The residue was taken up in CH_2Cl_2 (50 mL) and washed with 5% aqueous $NaHCO_3$ (50 mL), and the aqueous layer was extracted with CH_2Cl_2 (2×50 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated. Chromatography over silica (hexanes:ethyl acetate; 4:1) afforded compound **8** (1.49 g, 60%) as a yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.96 (1H, d, $J = 8.1$ Hz), 7.44 (1H, d, $J = 8.1$ Hz), 7.28 (4H, m), 6.90 (4H, m), 5.53 (1H, dd, $J = 5.1, 10.5$ Hz), 4.93 (1H, d, $J = 11.7$ Hz), 4.83 (2H, s), 4.76 (1H, d, $J = 11.7$ Hz), 4.56 (2H, m), 4.53 (1H, d, $J = 11.4$ Hz), 4.47 (1H, d, $J = 11.7$ Hz), 4.26 (1H, m), 4.14 (1H, m), 3.84 (6H, s), 3.65 (2H, m), 2.52 (1H, ddd, $J = 1.3$,

5.1, 13.2 Hz), 1.82 (1H, ddd, $J = 5.9$, 10.6, 13.5 Hz), 0.99 (9H, s), 0.92 (9H, s), 0.14 (6H, s), 0.13 (3H, s), 0.08 (3H, s). HRMS (MALDI) calcd for $C_{40}H_{61}NO_7Si_2$ (MH^+): 724.4065; found: 724.4087.

*2.2.7. {6-Hydroxymethyl-5-[4S-(4-methoxy-benzyloxy)-5R-(4-methoxy-benzyloxymethyl)-tetrahydro-furan-2R-yl]-pyridin-2-yl}-methanol (**9**)*

To a solution of compound **8** (2.07 g, 2.86 mmol) in THF (20 mL) at 0 °C was added a solution of tetrabutylammonium fluoride in THF (1.0 M, 11.5 mL, 11.5 mmol). After stirring for 4 h at RT, ethyl acetate (150 mL) and water (80 mL) were added to the reaction mixture, the phases were separated and the aqueous phase was extracted with ethyl acetate (2×80 mL). The combined organic extracts were washed with brine (80 mL), dried over $MgSO_4$ and concentrated. Chromatography over silica (CH_2Cl_2:MeOH:NEt_3; 100:8:0.5) afforded compound **9** (1.39 g, 98%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.08 (1H, d, $J = 7.7$ Hz), 7.36 (1H, d, $J = 8.1$ Hz), 7.25 (4H, m), 6.90 (4H, m), 5.21 (1H, dd, $J = 5.1, 10.6$ Hz), 4.91 (1H, m), 4.80 (1H, m), 4.51 (2H, d, $J = 3.3$ Hz), 4.49 (2H, s), 4.28 (1H, m), 4.16 (1H, d, $J = 6.2$ Hz), 3.83 (6H, s), 3.61 (2H, d, $J = 4.0$ Hz), 3.14 (4H, m), 2.38 (1H, dd, $J = 5.1, 12.8$ Hz), 1.83 (1H, ddd, $J = 5.9$, 10.6, 13.2 Hz). HRMS (MALDI) calcd for $C_{28}H_{33}NO_7$ (MH^+): 496.2335; found: 496.2339.

*2.2.8. 3-[4S-(4-Methoxy-benzyloxy)-5R-(4-methoxy-benzyloxymethyl)-tetrahydro-furan-2R-yl]-pyridine-2,6-dicarboxylic acid (**10**)*

To a solution of compound **9** (1.78 g, 3.59 mmol) in acetonitrile (18 mL) and phosphate buffer (pH 6.7, 13.5 mL) was added TEMPO (80 mg, 0.51 mmol) at RT. After warming to 35 °C a solution of sodium chlorite (1.65 g, 18.2 mmol) in water (3.6 mL) and a solution of sodium hypochlorite (1.00 g, 13.4 mmol) in water (1.8 mL) were added simultaneously over 1 h. After stirring at 35 °C overnight, water (25 mL) was added to the reaction mixture which was then adjusted to pH 8 by addition of 1 M NaOH. The resulting mixture was poured into an ice-cold saturated aqueous sodium thiosulfate solution (50 mL) and stirring was continued for 0.5 h. The pH was adjusted to pH 3 by slow addition of 1 M HCl (30 mL) and the aqueous phase was extracted with CH_2Cl_2 (6×100 mL). The combined organic extracts were washed with water (2×100 mL) and brine (2×100 mL), dried over $MgSO_4$ and concentrated to afford compound **10** (1.58 g, 84%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.51 (1 H, d, m), 8.25 (1H, m), 7.22 (4H, m), 6.84 (4H, m), 6.07 (1H, m), 4.52 (3H, m), 4.39 (1H, d, $J = 11$ Hz), 4.29 (1H, m), 4.10 (1H, m), 3.77 (3H, s), 3.75 (3H, s), 3.65 (2H, m), 2.87 (1H, m), 1.48 (1H, m). HRMS (MALDI) calcd for $C_{28}H_{29}NO_9$ (MNa^+): 546.1735; found: 546.1749.

*2.2.9. 3-[4S-(4-Methoxy-benzyloxy)-5R-(4-methoxy-benzyloxymethyl)-tetrahydro-furan-2R-yl]-pyridine-2,6-dicarboxylic acid dimethyl ester (**11**)*

To a solution of compound **10** (2.04 g, 3.88 mmol) in benzene (40 mL) and MeOH (10 mL) was added a 2.0 M solution of diazomethane in hexanes (5.5 mL, 11.0 mmol) at RT. Stirring was continued for 0.5 h and the resulting yellow solution was concentrated. Chromatography over silica (CH_2Cl_2:MeOH; 25:1) afforded compound **11**

(1.26 g, 59%) as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.33 (1H, d, $J = 8.4$ Hz), 8.16 (1H, d, $J = 8.4$ Hz), 7.23 (4H, m), 6.86 (4H, m), 5.69 (1H, dd, $J = 5.9, 9.9$ Hz), 4.51 (3H, m), 4.41 (1H, d, $J = 11.4$ Hz), 4.24 (1H, m), 4.09 (1H, m), 3.99 (3H, s), 3.97 (3H, s), 3.80 (3H, s), 3.79 (3H, s), 3.62 (2H, m), 2.72 (1H, ddd, $J = 2.2$, 6.2, 13.2 Hz), 1.74 (1H, ddd, $J = 6.2$, 9.9, 13.2 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 165.4, 165.0, 161.1, 159.2, 146.1, 145.6, 144.0, 136.7, 130.0, 129.4, 129.3, 127.5, 113.8, 84.1, 80.3, 76.7,73.1, 70.8, 70.4, 55.3, 53.1, 40.7. HRMS (MALDI) calcd for $C_{30}H_{33}NO_9$ (MH^+): 552.2228; found: 552.2225.

*2.2.10. 3-(4S-Hydroxy-5R-hydroxymethyl-tetrahydro-furan-2R-yl)-pyridine-2,6-dicarboxylic acid dimethyl ester (**12**)*

To a solution of compound **11** (1.26 g, 2.28 mmol) in CH_2Cl_2 (25 mL) was added a 20% solution of TFA in CH_2Cl_2 (25 mL) at RT. After stirring for 0.5 h at RT MeOH was added (100 mL) and the resulting mixture was concentrated and coevaporated with toluene (3×). Chromatography over silica (CH_2Cl_2:MeOH:NEt_3; 15:1:0.1) afforded compound **12** (700 mg, 98%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.23 (2H, s), 5.72 (1H, dd, $J = 5.9, 9.7$ Hz), 4.42 (1H, m), 4.04 (1H, m), 3.99 (3H, s), 3.97 (3H, s), 3.88 (1H, m), 3.82 (1H, m), 2.57 (1H, ddd, $J = 2.4$, 5.9, 13.4 Hz), 1.90 (1H, ddd, $J = 6.4$, 9.7, 13.2 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 165.9, 164.9, 146.3, 142.8, 136.2, 128.9, 127.4, 87.2, 76.1, 73.3, 63.2, 53.2, 44.1. HRMS (MALDI) calcd for $C_{14}H_{17}NO_7$ (MNa^+): 334.0897; found: 334.0907.

*2.2.11. 3-{5R-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-4S-hydroxy-tetrahydro-furan-2R-yl}-pyridine-2,6-dicarboxylic acid dimethyl ester (**13**)*

Compound **12** (700 mg, 2.25 mmol), azeotroped from pyridine (2 × 2 mL), was dissolved in pyridine (4 mL). Triethylamine (1.43 mL, 10.25 mmol), followed by DMTr-Cl (835 mg, 2.46 mmol) was added, and the reaction mixture was stirred overnight at RT. After addition of MeOH (5 mL), the solvent was removed. Chromatography over silica (50–66% ethyl acetate in hexanes, 1% NEt_3) afforded compound **13** (736 mg, 53%) as a white foam. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.36 (1H, d, $J = 8.1$ Hz), 8.15 (1H, d, $J = 8.3$ Hz), 7.43 (2H, m), 7.32 (4H, m), 7.26 (2H, m), 7.20 (1H, m), 6.81 (4H, m), 5.77 (1H, dd, $J = 6.2$, 9.2 Hz), 4.39 (1H, m), 4.09 (1H, m), 3.99 (3H, s), 3.96 (3H, s), 3.77 (6H, s), 3.38 (2H, m), 2.60 (1H, ddd, $J = 2.9$, 6.2, 13.2 Hz), 1.90 (1H, ddd, $J = 6.2$, 9.2, 13.2 Hz). HRMS (MALDI) calcd for $C_{35}H_{35}NO_9$ (MH^+): 614.6617; found: 614.6623.

*2.2.12. 3-{5R-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-4S-[(2-cyano-ethoxy)-diisopropylamino-phosphanyloxy]-tetrahydro-furan-2R-yl}-pyridine-2-6-dicarboxylic acid dimethyl ester (**1**)*

Compound **13** (405 mg, 0.66 mmol), azeotroped from pyridine (2 × 2 mL), was dissolved in CH_2Cl_2 (10 mL). *N*,*N*-Diisopropylethylamine (0.60 mL, 3.44 mmol) was added, followed by the dropwise addition of 2-cyanoethyl diisopropylamino-chloro phosphoramidite (0.31 mL, 1.39 mmol). After 3 h at RT, the reaction mixture was partitioned between CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ (50 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2

(2×50 mL). The combined organic phases were dried over $MgSO_4$ and concentrated. Chromatography over silica (33–50% ethyl acetate in hexanes, 1% NEt_3) afforded compound **2** (472 mg, 88%) as a white foam.

^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.40 (1H, m), 8.14 (1H, m), 7.44 (2H, m), 7.26 (7H, m), 6.80 (4H, m), 5.75 (1H, m), 4.48 (1H, m), 4.22 (1H, m), 4.00 (3H, s), 3.97 (3H, s), 3.85 (1H, m), 3.77 (6H, s), 3.60 (3H, m), 3.35 (2H, m), 2.66 (1H, m), 2.42 (1H, m), 1.90 (1H, m), 1.28 (1H, m), 1.18 (8H, m), 1.08 (4H, M). HRMS (MALDI) calcd for $C_{44}H_{52}N_3O_{10}P$ (MH^+): 814.3469; found: 814.3463.

2.3. Oligonucleotide synthesis

All oligonucleotides were prepared on a 1.0 μM scale. A standard protocol for 2-cyanoethyl phosphoramidites was used, except that the coupling of the phosphoramidites **1** and **dPy** was extended to 15 min. Phosphoramidites for phenoxacetyl protected dA (Pac-dA), 4-isopropyl-phenoxacetyl protected dG (*i*Pr-Pac-dG), and acetyl protected dC (Ac-dC) were used (ultramild synthesis reagents from Glen Research) and the oligonucleotides were deprotected for 6 h with 50 mM potassium carbonate in methanol at RT. The tritylated oligonucleotides were then purified by reversed phase HPLC (5–60% acetonitrile in 0.05 M aqueous TEAA, TEAA = tetraethyl ammonium acetate), detritylated with 80% acetic acid, and again purified by reversed phase HPLC (5–20% acetonitrile in 0.05 M aqueous TEAA). Identities of all oligonucleotides was confirmed by MALDI-TOF MS.

2.4. Thermal denaturation studies

All UV melting experiments were carried out in 5 mM sodium phosphate, 50 mM sodium perchlorate, pH 7.0, with 15 μM copper(II) acetate and 1 μM DNA duplex concentration. For the preparation of all samples and buffers analytical grade water free of metal ion contaminations (Fluka) was used. All samples were treated with Chelex-resin at RT overnight to remove Cu^{2+} contaminations prior to measurements. Melting temperature experiments were carried out on a Cary 300-Bio UV/Vis spectrophotometer. Melting curves were recorded at 260 nm for a consecutive heating (15–65 °C)-cooling-heating protocol with a linear gradient of 0.5 °C/min.

3. Results and discussion

3.1. Ligand design and synthesis

Based on the high pairing stability of our first-generation **Dipic**–**Py** metallo-base pair, we envisioned that small variations in the structure of the ligands might result in further improvements in pairing selectivity. We decided to further optimize the ligand structure of the tridentate **Dipic** ligand without changing the structure of the monodentate **Py** ligand. Phosphoramidite **1** serves as a precursor for the synthesis of the **Dipic** ligand. After incorporation of this phosphoramidite into oligonucleo-

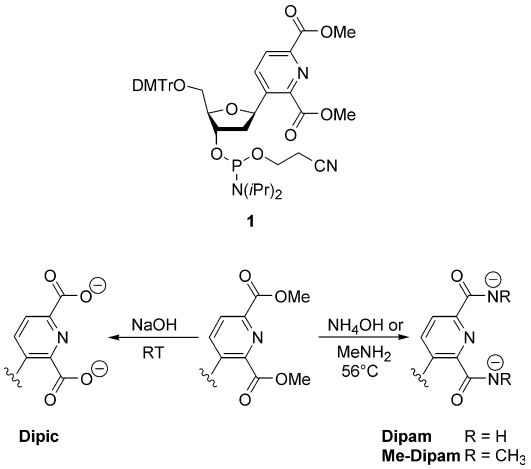


Fig. 2. Structure of phosphoramidite **1** and, after incorporation into oligonucleotides, conversion to **Dipic** by treatment with NaOH or nucleobases **Dipam** and **Me-Dipam** by treatment with NH_4OH or $MeNH_2$.

tides using a DNA synthesizer, the two dimethylester groups were saponified to give the two metal-coordinating carboxylate groups of the **Dipic** ligand (Fig. 2). By using a convertible nucleoside approach we replaced the ester groups of this same precursor with two different amide groups: the two carboxylate groups of the *O*, *N*, *O*-**Dipic** ligand (X = O) were replaced with amide substituents to afford the two new *N*,*N*,*N*-ligand systems **Dipam** (X = NH) and **Me-Dipam** ($X = NCH_3$) (Fig. 2). In the case of pyridine-containing ligands with amide side chains it has been reported that complexation of Cu^{2+} probably occurs through the deprotonated amide *N*-atom under neutral conditions [9]. Since the deprotonated amide group and the carboxylate group are isoelectronic, we expected very similar electronic properties of both new ligand systems. On the other hand the steric properties differ since **Dipam** and **Me-Dipam** contain additional substituents (H or Me, respectively) on the coordinating N atom which might result in different pairing stability and/or pairing selectivity.

The synthetic route for phosphoramidite **1** is shown in Scheme 1. Compound **5** was synthesized in five steps starting from 2,6-lutidine. Lithiation of **5** followed by nucleophilic addition to *aldehydo*-D-ribose **6** [8] gave a diastereomeric mixture of alcohols **7**(**1R**) and **7**(**1S**) in equal amounts. After separation of the diastereomers by chromatography, **7**(**1S**) was cyclized to give the β-nucleoside **8**. Cleavage of the TBDMS-ethers followed by oxidation and esterification afforded compound **11**. The PMB protecting groups were removed, and tritylation followed by phosphitylation provided phosphoramidite **1** which was then incorporated into the middle of a 15-nucleotide strand using an automated DNA synthesizer. Treatment of the oligonucleotide with concentrated ammonium hydroxide or methylamine at 56 °C for 12 h afforded the nucleobases **Dipam** and **Me-Dipam** in 85% and 84% yield, respectively [10]. The identities of the oligonucleotides were confirmed by MALDI-TOF spectroscopy.

3.2. Base pairing stability and selectivity

Our first generation **Dipic–Py** metallo-base pair shows high pairing stability but lacks selectivity. When introduced into the middle of the 15-nucleotide duplex **14**, the duplex displays almost comparable melting temperatures (T_m) in the presence of Cu^{2+} to a duplex with a dA:dT pair at the same site ($T_m = 36.5$ and 39.1 °C for **Dipic:Py** and dA:dT, respectively, Table 1) [11]. However, the range in T_m of the **Dipic** base when paired with natural bases is 26.2–34.1 °C. Relatively stable mispairs are formed with dA and dG; **Dipic**:dA forms the most stable mispair and is destabilized by only 2.4 °C relative to **Dipic:Py**. For comparison, mispairs between the natural nucleobases are 9.6–15.8 °C less stable in this sequence context. Nucleobase **Dipam** (X = NH) shows both a very high pairing stability and selectivity. In the presence of fifteen equivalents Cu^{2+} ions, the **Dipam:Py** base pair is even more stable than a dG:dC and a dA:dT base pair ($T_m = 43.0$, 40.1 and 39.1 °C for **Dipam:Py**, dG:dC and dA:dT, respectively, Table 1) [12]. Like the **Dipic** nucleobase, the purine nucleobases dA and dG still form the most stable mispairs with **Dipam** but the base pairing selectivity has been significantly enhanced: the most stable mispair (**Dipam**:dG) is destabilized by 8.5 °C relative to **Dipam:Py**, and the **Dipam**:dA mispair is destabilized by 13.0 °C. Although the most stable mispair is with dA for **Dipic** and with dG for **Dipam**, the absolute values of the melting temperatures remain almost the same (34.1 and 34.5 °C for **Dipic**:dA and **Dipam**:dG, respectively). Since the pairing stability of **Dipam**:Py exceeds the stability of the natural dA:dT and dC:dG base pairs, pairing selectivity is gained at the same time. Thus the **Dipam:Py** base pair exhibits pairing stability and selectivity comparable to the natural base pairs. The rationale for these improved properties of the **Dipam:Py** metallo-base pair over

Table 1
Sequence of the 15-mer DNA duplex **14** and denaturation temperatures (T_m) for various combinations of X and Y[a]

Y	X						
	Dipam	**Dipic**	A	T	C	G	**Me-Dipam**
Dipam	[b]	n.d.[c]					
Dipic	n.d.[c]	[b]					
Py	43.0 (28.0)[d]	36.5	23.6	29.5	23.5	25.7	26.5 (26.6)[d]
A	30.0	34.1	[b]	39.1	24.0	27.7	
T	27.5	[b]	39.1	27.6	26.1	30.5	
C	25.5	26.2	23.3	27.3	[b]	40.1	
G	34.5	28.6	28.1	29.5	39.5	[b]	

[a] Melting temperature experiments were carried out on a Cary 300-Bio UV/Vis spectrophotometer. The heating rates were 0.5 °C min^{-1}. Conditions: 5 mM sodium phosphate, 50 mM sodium perchlorate, pH 7, with 15 M copper(II) acetate and 1 μM DNA duplex (5′-CACATTA**X**TGTTGTA-3′ and 3′-GTGTAAT**Y**ACAACAT-5′ (**14**)

[b] No sigmoidal melting curve was observed in the temperature range between 15 and 65 °C.

[c] Not determined.

[d] Melting temperature in the absence of copper(II) acetate.

the **Dipic:Py** remains unclear at this point. Efforts are underway to obtain a structure of a DNA duplex containing the **Dipam:Py** metallo-base pair to gain further insight into the increased pairing selectivity and stability of this metallo-base pair. X-ray analysis would also clarify the exact coordination mode of the amide groups to the metal center since coordination through the N-atoms is more likely but coordination through the O-atoms cannot be excluded at this point. UV-melting experiments revealed that nucleobase **Me-Dipam** ($X = NCH_3$) does not form a stable metallo-base pair with the **Py** nucleobase, as there is no change in T_m in the presence of Cu^{2+} (Table 1). The two methyl substituents of the amide groups might be sterically too demanding to properly fit into a DNA duplex. This might increase the C1′–C1′ distance to an extent that metal-coordination of the **Py** base to the Cu(II) ion becomes less effective which might result in the loss of pairing stability. In addition, the p*K* value of the **Me-Dipam** ligand is likely to be shifted to a higher value in comparison to the **Dipam** ligand due to the introduction of the methyl groups. This change in p*K* value makes the deprotonation of the amide group more difficult which might result in destabilization of the corresponding metal complex.

3.3. DNA containing multiple metallo-base pairs

Metallo-base pairing in combination with automated DNA synthesis offers a convenient way to construct DNA duplexes containing metal ions in defined locations [13]. The construction of oligomers consisting of multiple metallo-base pairs could lead to materials with interesting electronic properties based on the controlled and periodic spacing of metal ions along the helix axis. The high pairing stabilities of both the **Dipam:Py** and the **Dipic:Py** base pair prompted us to study the UV-melting behaviour of duplexes containing multiple metallo-base pairs. Fifteen-nucleotide duplexes with two and four adjacent metallo-base pairs (**16a/b–17a/b**) were synthesized and their thermal stability in the presence of 15 equivalents of Cu^{2+} ions per metallo-base pair was determined (Fig. 3) [14,15]. In duplexes **16a/b** one dA:dT base pair adjacent to the metallo-base pair of duplexes **15a/b** was replaced by a second metallo-base pair. This substitution resulted in an increased duplex stability of 0.4/2.0 °C (43.0/43.4 °C for **15a/16a** and 36.5/38.5 °C for **15b/16b**, respectively) in comparison to duplexes **15a/b** containing one metallo-base pair. Further substitution

5'-CACATTA**P**TGTTGTA-3'	**15a**	**D = Dipam**,	43.0 °C
3'-GTGTAAT**D**ACAACAT-5'	**15b**	**D = Dipic**,	36.5 °C
5'-CACATT**DP**TGTTGTA-3'	**16a**	**D = Dipam**,	43.4 °C
3'-GTGTAA**PD**ACAACAT-5'	**16b**	**D = Dipic**,	38.5 °C
5'-CACAT**PDDP**GTTGTA-3'	**17a**	**D = Dipam**,	47.3 °C
3'-GTGTA**DPPD**CAACAT-5'	**17b**	**D = Dipic**,	39.6 °C

Fig. 3. Fifteen-nucleotide duplexes containing adjacent metallo-base pairs (**P = Py**, **D = Dipam** or **Dipic**) and their stabilities determined by melting temperature experiments.

of two more dA:dT base pairs with two metallo-base pairs gave duplexes **17a/b** containing four adjacent metallo-base pairs. In comparison to duplexes **16a/b** with two metallo-base pairs, duplex stabilities were further increased by 3.9/1.1 °C (43.4/47.3 °C for **16a/17a** and 38.5/39.6 °C for **16b/17b**, respectively). These results demonstrate that natural base pairs can be efficiently replaced with metallo-base pairs at multiple sites in DNA duplexes. Efforts are underway to investigate the potentially novel electronic properties of these DNA duplexes containing multiple metallo-base pairs.

4. Conclusions

In summary we have generated an improved copper(II)-mediated metallo-base pair with a high pairing stability and selectivity: the pyridine-2,6-dicarboxamide–pyridine metallo-base pair (**Dipam:Py**) is more stable than the natural dC:dG and dA:dT base pairs, and shows high selectivity against mispairing with the natural bases. Construction of stable DNA duplexes containing multiple metallo-base pairs demonstrates that hydrogen bonding of natural base pairs can efficiently be replaced with copper(II)-mediated metallo-base pairing. Efforts are underway to investigate the electronic properties of DNA duplexes containing multiple metallo-base pairs.

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[10] Yield is given after purification by reversed phase HPLC. Identities were confirmed by MALDI-TOF MS. 15-mer oligonucleotides containing **Dipic-Ester** or **Me-Dipam** have the same mass but could be distinguished by their different retention times using reversed phase HPLC.

[11] In [6a] slightly higher T_m-values were reported. T_m-values reported herein were confirmed by repeated experiments and were consistent under the experimental conditions reported here.

[12] In comparison to the **Dipic:Py** metallo-base pair, the **Dipam:Py** base pair required the presence of at least five equivalents of Cu^{2+} ions for the formation of a stable duplex. Interestingly, the **Dipam:Py** base pair forms a slightly stable base pair even in the absence of Cu^{2+} ions ($T_m = 28.0$ °C) which is not the case for the **Dipic:Py** base pair. This stabilizing effect may be caused by weak hydrogen bonding interactions. It might also explain the observed difference in required metal ion concentrations of both metallo-base pairs due to different kinetics for the formation of the corresponding metal complexes.

[13] Shionoya and coworkers recently reported a self-assembled metal array in artificial DNA consisting of copper(II)-mediated base pairs between hydroxypyridone nucleobases: K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shionoya, Science 299 (2003) 1212–1213.

[14] Conditions: 5 mM sodium phosphate, 50 mM sodium perchlorate, pH 7, 1 μM DNA duplex, 30 μM (**15a/b**)/60 μM (**16a/b**) copper(II) acetate..

[15] No sigmoidal melting curves were observed for duplexes **16a/b** and **17a/b** in the absence of Cu^{2+} ions.