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Phosphoramidites and solid supports based on *N*-substituted 2,4-dihydroxybutyramides: universal reagents for synthesis of modified oligonucleotides

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Abstract—A general and convenient method for synthesis of modified oligonucleotides by use of new non-nucleoside phosphoramidites is reported. A chiral 1,3-diol backbone of the modifying reagents is generated either from (*R*)-(+)-α-hydroxy-γ-butyrolactone or (*R*)-(−)-pantolactone. Aliphatic amines were acylated with the lactones to give the corresponding *N*-substituted 2,4-dihydroxybutyramides. After protection of a side chain, if necessary, the diols were converted into phosphoramidites or solid supports suitable for use in oligonucleotide synthesis. The reagents allow single, multiple or combined introduction of various functions (e.g., alkylamine, imidazole and pyrene residues) into synthetic oligonucleotides. The structures of the conjugates were confirmed by MALDI-TOF mass spectrometry.

1. Introduction

Although modified oligonucleotides have diverse use in chemistry, biology and medicine, the emergence of new areas of their application is a current trend.[1] Solid-phase phosphoramidite oligonucleotide synthesis, a highly efficient and reliable technique developed in the 1980s,[2] is still a mainstay of chemical DNA preparation. Numerous modifying phosphoramidite reagents for oligonucleotide synthesis also have been developed[3] and many of them are commercially available. The potential of phosphoramidites for combinatorial chemistry has been recognised.[4] Our aim was to develop reagents applicable for the preparation of oligonucleotide conjugates in combinatorial mode, for instance, for synthesis of artificial ribonucleases that have an oligonucleotide part for complementary recognition and a variable catalytic part carrying active functional groups, e.g., amine, imidazole, etc.

A 1,3-diol system with one primary and one secondary hydroxyl group mimicking the 5′- and 3′-OH groups of nucleosides is an obvious structural element in the design of non-nucleoside reagents suitable for machine-aided assembly of modified oligonucleotides. We recently reported that it can be obtained easily by acylation of amines with α-hydroxy-γ-butyrolactones.[5] In the procedure, a suitable compound with an aliphatic primary or even secondary amino group can be converted into the corresponding *N*-substituted 2,4-dihydroxybutyramide. The precursor is then subjected to successive steps of side-chain protection (if required), 4,4′-dimethoxytritylation of the primary hydroxyl group, and conversion into the phosphoramidite or attachment to a solid support via the secondary hydroxyl group. Here we describe the synthesis of several new reagents that allow the introduction of reactive (amine), catalytically active (imidazole) and fluorescent (pyrene) residues into oligonucleotides as well as the preparation and characterisation of various oligonucleotide conjugates.

2. Results and discussion

2.1. Synthesis of phosphoramidites

α-Hydroxy-γ-butyrolactones **1a**,**b** are known to react smoothly with amines.[6] No racemisation at the α-carbon

Keywords: Modified oligonucleotides; Phosphoramidites; 2,4-Dihydroxybutyramides; Imidazole; Pyrene.
Abbreviations: DIC, 1,3-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; Dmt, 4,4′-dimethoxytrityl; Fmoc, 9-fluorenylmethoxycarbonyl; FmocOSu, 9-fluorenylmethyl *N*-succinimidyl carbonate; LCAA-CPG, long-chain alkylamino controlled pore glass; Py, pyridine; RP-HPLC, reversed-phase HPLC; Tfa, trifluoroacetyl.
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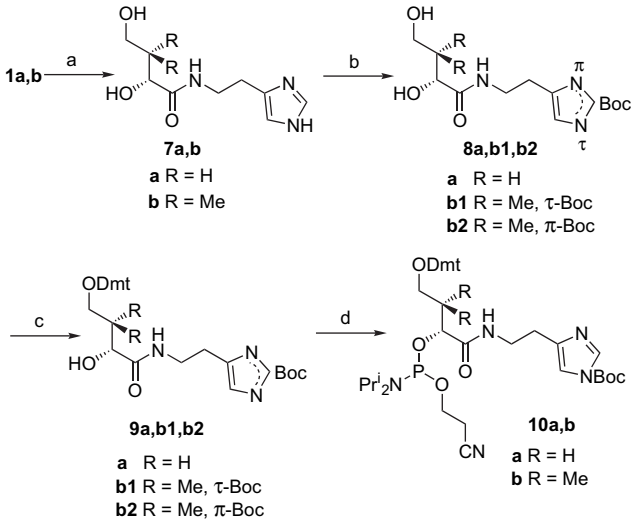
atom was reported. We found that in the case of α-hydroxy-γ-butyrolactones **1a** and **1b** a simple un-catalysed reaction with primary amines at a slightly elevated temperature gives very good yields of the corresponding 2,4-dihydroxybutyramides after 24–48 h. Primary amines with a bulky substituent, e.g., pyrene or secondary amine, e.g., *N*,*N*′-dimethylethylenediamine require much longer reaction times.

α,ω-Diamines served as precursors for aminolinker reagents. Lactones **1a** and **1b** were treated with an excess (5.0 equiv) of ethylenediamine or *N*,*N*′-dimethylethylenediamine at 55 °C for 1–7 days to give mono-acylated products **2** (Scheme 1).

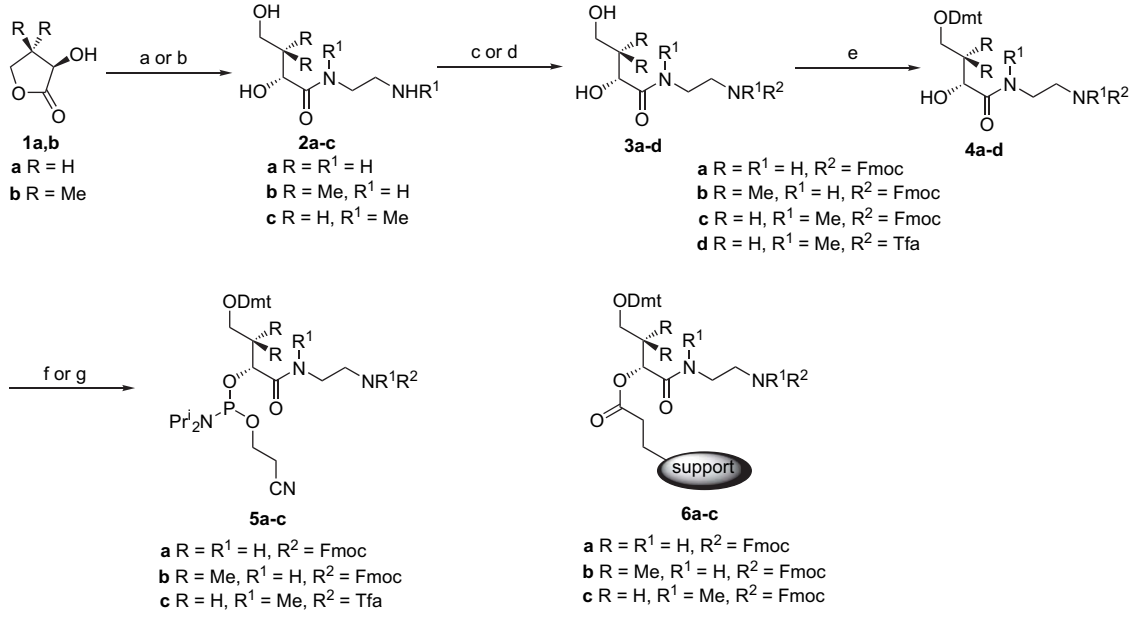
The remaining amino function was masked with Fmoc or Tfa, common amino protecting groups compatible with oligonucleotide synthesis,[3] affording **3a–d**. Compound **3a** precipitated readily from the reaction mixture. By contrast, the presence of two geminal 3-methyl groups in the pantolactone-derived compound **3b** or N-methylation in **3c**,**d** allow their purification by column chromatography. The hydroxyl groups in compounds **3** were functionalised by known procedures of nucleoside chemistry:[7] the primary hydroxyl group was 4,4′-dimethoxytritylated[8] selectively, and the resulting compounds **4** were converted into phosphoramidites **5a–c**[9] and solid-supported reagents **6a–c**.[10] The loading of the supports was estimated to be in the range of 35–52 μmol/g. Phosphoramidites were found to decompose significantly on silica gel during column chromatography and thus were isolated by precipitation into hexane. Reagents **5a–c** are white solids that can be stored at −20 °C for at least several months without any substantial loss of reactivity. Phosphoramidite **5b** is appreciably more stable upon storage compared to **5a**, which indicates a positive shielding effect of the two methyl groups. The reagents allow introduction of one or several primary or secondary amino groups into any position within an oligonucleotide sequence.

Imidazole residues of histidines are present in catalytic centres of DNA- and RNA-cleaving enzymes.[11] Many syntheses of imidazole–oligonucleotide conjugates as potential target-addressed RNA cleavers are reported.[12] It was found that the Boc group is suitable for histidine N^{Im}-protection during phosphoramidite DNA synthesis, undergoing smooth cleavage upon standard ammonolytic deprotection conditions.[13] This prompted us to investigate its use for imidazole protection in phosphoramidite reagents **10a**,**b**.

The synthesis of the imidazole reagents is outlined in Scheme 2. The primary amino group of histamine was acylated with lactones **1a** or **1b** to give dihydroxybutyramide **7a**



Scheme 2. Synthesis of imidazole phosphoramidites. Reaction conditions and yields: (a) histamine, 55 °C, 83% (**7a**); (b) Boc_2O, 76% (**8a**), 38% and 17% (**8b1** and **8b2** from **1b**); (c) DmtCl, Py, 0 °C to rt, 1–3 h, 86% (**9a**), 55% and 37% (**9b1** and **9b2** from **8b1**); (d) $(^{i}Pr_2N)_2PO(CH_2)_2CN$, diisopropylammonium tetrazolide, DCM, 80% (**10a**), 60% (**10b**).



Scheme 1. Synthesis of amino-modifier reagents and solid supports. Reaction conditions and yields: (a) $(CH_2NH_2)_2$, 55 °C, overnight, quant. (**2a–b**); (b) $(CH_2NHMe)_2$, 55 °C, 7 days, quant. (**2c**); (c) FmocOSu, aq MeCN, 88% (**3a**), 75% (**3b**), 64% (**3c**); (d) CF_3CO_2Me, rt, 30 min; (e) DmtCl, Py, 0 °C to rt, 1–3 h, 81% (**4a**), 97% (**4b**), 89% (**4c**), 93% (**4d** from **2c**); (f) $(^{i}Pr_2N)_2PO(CH_2)_2CN$, diisopropylammonium tetrazolide, DCM, rt, 2 h, 85% (**5a**), 67% (**5b**), 77% (**5c**); (g) succinylated LCAA-CPG, DIC, DMAP, Py, rt, loading (μmol/g): 35 (**6a**), 52 (**6b**), 44 (**6c**).

or **7b**, respectively. These were treated with Boc_2O to afford imidazole-protected compounds **8**. Only the τ-Boc isomer was isolated in the case of **8a**, whilst **7b** gave a mixture of τ- and π-isomers (**8b1** and **8b2**), easily separable by column chromatography. Dimethoxytritylation of **8** followed by phosphitylation yielded phosphoramidites **10**. Only the major isomer **8b1** was used in the reaction with DmtCl in pyridine.

Surprisingly, two separable products **9b1** and **9b2** were again isolated after a usual workup and column chromatography. This indicates that partial τ→π migration of the Boc group takes place during dimethoxytritylation, possibly catalysed by pyridine hydrochloride formed in the reaction. π-Isomers (**8b2** and **9b2**) were found to be unstable: after storage of the compounds as dried solids for one month at +4 °C, **8b2** gave ca. 1:1 mixture of **8b1** and **8b2**, and **9b2** was completely converted into **9b1**. The structures of compounds **8b1**, **8b2** and **9b1** were determined using Rapoport's NMR criteria.[14,15] Moreover, a ROESY experiment for **9b1** (spectral width 10 ppm×10 ppm, 16,000×512 of complex points) showed similar cross-peaks of medium intensity between Boc protons and both H-2 and H-5 of the imidazole moiety.

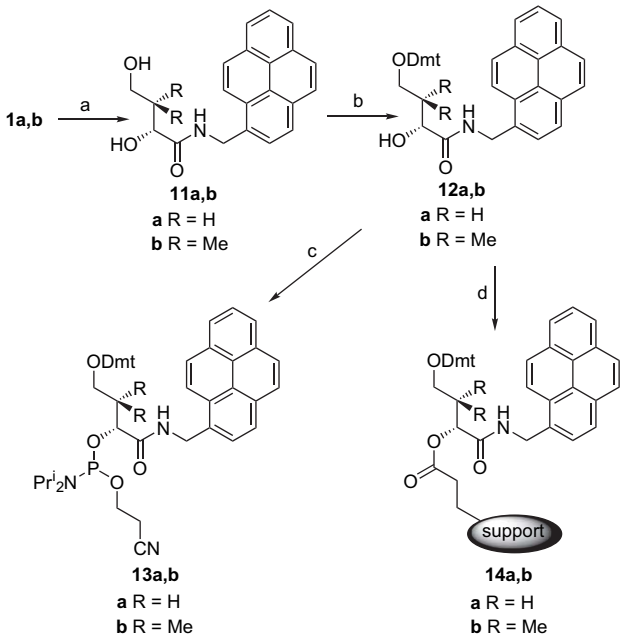
Interestingly, the less stable π-Boc isomers and their rearrangement to thermodynamically more stable τ-Boc compounds were detected in the '**b**' series only. The influence of the remote *gem*-dimethyl group in **7b** on regioselectivity of imidazole acylation in comparison with **7a** seems to be unlikely. The observed difference could be explained rationally by the following assumptions: (1) π-Boc isomer is also formed from **7a**, but converts rapidly into the stable τ-Boc compound; (2) the substantial difference in π→τ-migration rates between **a** and **b** series is caused by spatial hindrance of the hydroxyl(s) that probably facilitates the rearrangement.

Compound **9b1** was then phosphitylated to give **10b**, a pair of diastereomers arising from the phosphoramidite group, as a single product. No migration of the Boc group was observed during the reaction.

Of course, the exact position of the Boc group in the reagents **10a**,**b** does not influence the structure of the resultant imidazole–oligonucleotide conjugates, since both τ- and π-Boc isomers should be deprotected in concd aq ammonia.

To the best of our knowledge, amongst pyrene non-nucleoside reagents described to date, only a few examples contain a 1,3-diol backbone.[16] Multistep syntheses of deoxyribosyl pyrene *C*-nucleosides are complex, time consuming and include separation of a mixture of α- and β-anomers.[17] Scheme 3 depicts a straightforward synthesis of new pyrene phosphoramidite reagents **13a**,**b** and supports **14a**,**b** from 1-pyrenemethylamine.

The structures of 2,4-dihydroxybutyramide derivatives **2a**, **3a**,**b**, **7a**, **8a**,**b1** and **11a**,**b** were confirmed by ^{1}H–^{13}C NMR correlations. The assignment of signals in the ^{13}C NMR spectra was performed using HMQC (cross-peaks from proton-bound carbon atoms) and HMBC (cross-peaks from interaction through two, three and four bonds) spectra.



Scheme 3. Synthesis of pyrene phosphoramidites. Reaction conditions and yields: (a) 1-pyrenemethylamine, 55 °C, 100% (**11a**), 92% (**11b**); (b) DmtCl, Py, 62% (**12a**), 83% (**12b**); (c) $(^{i}Pr_2N)_2PO(CH_2)_2CN$, diisopropylammonium tetrazolide, DCM, 70% (**13a**), 92% (**13b**); (d) succinylated LCAA-CPG, DIC, DMAP, pyridine, rt, loading (μmol/g): 20 (**14a**), 16 (**14b**).

2.2. Synthesis and characterisation of modified oligonucleotides

All the phosphoramidites and supports obtained were tested in machine-assisted solid-phase oligonucleotide synthesis. The coupling time for the modified phosphoramidites **5a**–**c**, **10a**,**b** and **13a**,**b** was extended to 10 min. The coupling yields were comparable to those for nucleoside phosphoramidites (determined by the released Dmt cation). The conjugates prepared were cleaved from their supports, deprotected by concd aq ammonia treatment at 55 °C overnight, analysed by reversed-phase HPLC and MALDI-TOF MS and purified by PAGE for thermal denaturation studies. Some loss of the 5′-terminal modified unit(s) was observed when the Dmt group was removed prior to ammonolysis, possibly resulting from the attack of the 5′-terminal hydroxyl on the neighbouring phosphodiester bond. To obtain uniformly high yields of the 5′-modified oligonucleotides these were synthesised routinely in 'Dmt On' mode, and the Dmt protecting group was cleaved after ammonia treatment. Sequences and some properties of the modified oligodeoxyribonucleotides are summarised in Table 1. Examples of HPLC traces are shown in Figure 1. Even in the crude mixtures after synthesis, the content of the desired conjugates was usually more than 90% with almost no detectable amount of any side products.

As expected, the incorporation of the hydrophobic pyrene moiety into oligonucleotides increases the retention time of conjugates on the HPLC column whilst other 2,4-dihydroxybutyramide substituents influence the chromatographic mobility of the corresponding oligomers only negligibly.

Thermal stability experiments show, as expected,[18] an increase in T_m for duplexes with pyrene 2,4-dihydroxybutyramide in

Table 1. Properties of oligodeoxyribonucleotides bearing (*R*)-2,4-dihydroxybutyramide non-nucleoside units

#	Sequence, 5′ to 3′[a]	Amidite or support source of X	MALDI-TOF, found/calcd	RP-HPLC retention time, min[b]
ON01	CTCCCAGGCTCAAAT**X**p	**5a**	4801.1/4796.8	15.20
ON02	CTCCCAGGCTCAAAT**X**p	**5b**	4824.8/4826.9	12.68
ON03	**X**CTCCCAGGCTCAAAT	**5b**	4750.8/4749.2	12.48
ON04	CTCCCAGGCTCAAAT**X**	**6a**	4718.7/4719.2	12.75
ON05	CTCCCAGGCTCAAAT**XX**	**6b**, **5b**	5078.8/5078.9	13.23
ON06	CTCCCAGGCTCAAAT**X**p	**13a**	4970.1/4969.9	22.21
ON07	**X**CTCCCAGGCTCAAAT**X**	**13b**, **14b**	5341.7/5341.0	34.80
ON08	CTCCCAGGCTCAAAT**X**	**14a**	4890.5/4889.9	24.85
ON09	CTCCCAGGCTCAAAT**X**	**14b**	4920.6/4920.3	27.71
ON10	ATTTGAGCCTGGGAG**X**	**14a**	5041.9/5040.9	21.42
ON11	ATTTGAGCCTGGGAG**X**	**14b**	5070.4/5071.4	25.30
ON12	CTCCCAGGCTCAAAT**X**	**6b**	4747.9/4749.2	12.51
ON13	CTCCCAGGCTCAAAT**X**p	**10a**	4851.8/4850.8	12.79
ON14	**X**CTCCCAGGCTCAAAT	**10a**	4773.5/4772.1	12.63
ON15	CTCCCAGGCTCAAAT**X**p	**10b**	4888.9/4881.2	12.55
ON16	**X**CTCCCAGGCTCAAAT	**10b**	4804.7/4801.2	13.17
ON17	CTCCCAGGCTCAAAT**XX**p	**10a**	5124.6/5124.9	12.52
ON18	**XX**CTCCCAGGCTCAAAT	**10a**	5044.2/5044.9	12.54
ON19	CTCCCAGGCTCAAAT**XX**p	**10b**	5182.8/5183.4	13.02
ON20	**XX**CTCCCAGGCTCAAAT	**10b**	5102.5/5103.5	14.14
ON21	CTCCCAGGCTCAAAT**X**	**6c**	4750.3/4749.2	13.78
ON22	CTCCCAGGCTCAAAT**X**p	**5c**	4847.3/4844.1	12.90
ON23	**X**CTCCCAGGCTCAAAT	**5c**	4748.0/4749.2	12.64
ON24	CTCCCAGGCTCAAAT**XX**p	**5c**	5081.7/5080.0	13.26
ON25	**XX**CTCCCAGGCTCAAAT	**5c**	5001.7/5001.4	12.71
ON26	**X**CTCCCAGGCTCAAAT**X**p	**5c**	5079.0/5080.0	12.59
ON27	**CUCCCAXGCUCA**[c]	(**5a**+**10a**+dG)	3731.7/3729.1 3784.5/3780.0 3836.7/3833.2	—[d]
ON28	**CUCCCAXGCUCA**[c]	(**5b**+**10b**+2′OMe-rG)	3761.5/3762.5 3812.6/3814.1 3868.6/3873.3	—[d]

[a] p—3′-phosphate.
[b] HPLC conditions are given in Section 4.
[c] 2′-*O*-Methylribonucleotides are in bold.
[d] Broad unresolved peak.

a dangling position and a slight destabilisation for duplexes with the 3′-phosphate group (Table 2). Other terminal modifications influence the T_m values insignificantly.

Next, we tested the suitability of 2,4-dihydroxybutyramide reagents for the generation of combinatorial libraries of oligonucleotides. We used a 1:1:1 (mol) mixture of **5a** and **10a** with dG phosphoramidite for synthesis of **ON27** and **5b** and **10b** with 2′-OMe-rG phosphoramidite in the same ratio for synthesis of **ON28**. The sum of the concentrations of phosphoramidites was 0.1 M in acetonitrile. Oligonucleotides were synthesised in 'Dmt On' mode and purified by RP-HPLC (for conditions see Section 4). The purified Dmt-containing oligomers were treated with 80% acetic acid at room temperature for 30 min, isolated by 1 M $LiClO_4$–acetone precipitation, and analysed by RP-HPLC and MALDI-TOF MS. In both cases, HPLC showed only one broad peak at ca. 15 min (data not shown). In the mass spectra, three main signals corresponding to individual library members were present (Fig. 2).

The spectra indicate that the reactivity of phosphoramidites derived from 2,4-dihydroxybutyramide is similar to 2′-deoxyribonucleoside phosphoramidites, and of those from 2,4-dihydroxy-3,3-dimethylbutyramide to 2′-*O*-methylribonucleoside phosphoramidites.

3. Conclusions

Our results demonstrate that various primary and secondary aliphatic amines may be easily converted into 2,4-dihydroxybutyramide phosphoramidites and solid supports and used for the preparation of modified oligonucleotides carrying diverse functional groups, e.g., amine, imidazole or pyrene. When a mixture of modifying phosphoramidites is used, a mixture of conjugates is produced that shows the potential of the reagents for application in combinatorial chemistry.

4. Experimental

4.1. General

500 MHz ^{1}H, 125.7 MHz ^{13}C and 202.4 MHz ^{31}P NMR spectra were recorded on a Bruker DRX-500 spectrometer and referenced to DMSO-d_6 (2.50 ppm for ^{1}H and 39.70 ppm for ^{13}C), MeCN-d_3 (1.96 ppm for ^{1}H), MeOH-d_3 (3.34 ppm for ^{1}H and 49.00 ppm for ^{13}C) and 85% aq H_3PO_4 (0.00 ppm for ^{31}P). ^{1}H–^{13}C gradient-selected HMQC and HMBC spectra were obtained by using 2048 (t_2)×256 (t_1) complex point data sets, zero filled to 2048 (F_2)×1024 (F_1) points. The spectral widths were 13 and 200 ppm for ^{1}H and ^{13}C dimensions, respectively. HMBC spectra were measured with 50 ms delay for evolution of long-range couplings. Varian

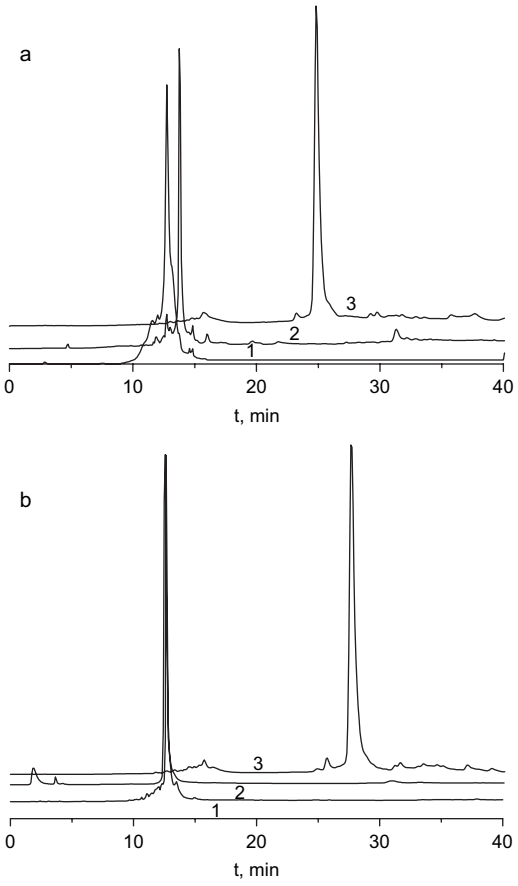


Figure 1. (a) Typical RP-HPLC traces of crude (*R*)-2,4-dihydroxybutyramide-containing oligodeoxyribonucleotides: (1) 2-aminoethyl **ON04**, (2) *N*,*N*′-dimethyl-2-aminoethyl **ON21** and (3) 1-pyrenemethyl **ON08** (Table 1). (b) Typical RP-HPLC traces of crude (*R*)-2,4-dihydroxy-3,3-dimethylbutyramide-containing oligodeoxyribonucleotides: (1) 2-aminoethyl **ON02**, (2) 2-(imidazol-4′-yl)ethyl **ON15** and (3) 1-pyrenemethyl **ON09** (Table 1). For HPLC conditions, see Section 4.

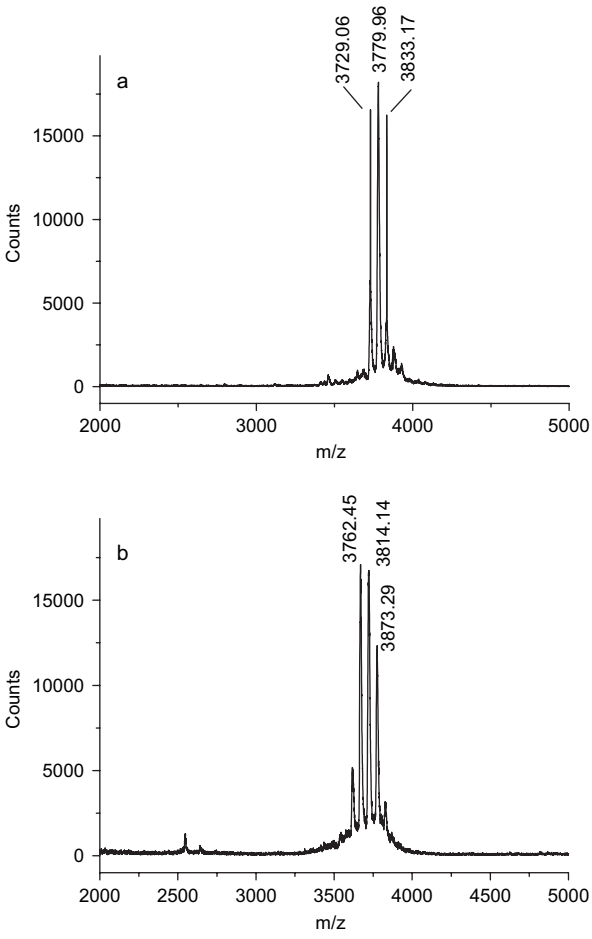


Figure 2. MALDI-TOF mass spectra of combinatorial mixtures **ON27** (a) and **ON28** (b).

Unity NMR spectrometer (600 MHz) was used for measuring DQF-COSY and ROESY spectra. ^{1}H NMR coupling constants are reported in Hertz and refer to apparent multiplicities. ESI-TOF HRMS spectra in positive ion mode were recorded on Micromass LCT reflection TOF mass spectrometer. IR spectra were recorded using Bruker Vector 22 spectrometer. Melting points were determined using a Boetius heating table and are uncorrected. Analytical thin-layer chromatography was performed on Kieselgel 60 F_{254} precoated aluminium plates (Merck), spots were visualised under UV light (254 nm). Silica gel column chromatography was performed using Merck Kieselgel 60 0.040–0.063 mm.

Table 2. Stability of various (*R*)-2,4-dihydroxybutyramide-modified oligodeoxyribonucleotides as duplexes with an unmodified complementary DNA strand

#	Modifying reagent	Position of modification	T_m, °C	ΔT_m/mod, °C
ON04	**6a**	3′-	57.3	−0.3
ON08	**14a**	3′-	61.6	+4.0
ON09	**14b**	3′-	62.0	+4.4
ON10	**14a**	3′-	59.1	+1.5
ON11	**14b**	3′-	58.5	+0.9
ON13	**10a**	3′-	57.4	−0.2
ON15	**10b**	3′-	57.3	−0.3
ON16	**10b**	5′-	58.9	+1.3
ON17	**10a**	3′-	56.9	−0.4
ON18	**10a**	5′-	57.9	+0.2
ON19	**10b**	3′-	56.9	−0.4
ON20	**10b**	5′-	58.0	+0.2
ON22	**5c**	3′-	57.0	−0.6
ON23	**5c**	5′-	58.1	+0.5
ON24	**5c**	3′-	56.7	−0.5
ON25	**5c**	5′-	58.4	+0.4
ON26	**5c**	5′- and 3′-	56.7	−0.5

Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. Diisopropylammonium tetrazolide was prepared as described.[19] DCM was used freshly distilled from CaH_2. THF was freshly distilled from powdered $LiAlH_4$ and stored over 4 Å molecular sieves under argon. Other solvents were used as received.

Oligonucleotide synthesis was carried out on a ABI 380B DNA/RNA synthesiser either on 0.2 or 1 μmol scale using 2′-deoxynucleoside phosphoramidites from Cruachem (Scotland). The molecular mass of each oligonucleotide was checked by MALDI-TOF MS on a Perseptive Biosystems Voyager DE workstation in positive ion mode using a mixture (1:1 v/v) of 2,6-dihydroxyacetophenone (40 mg/mL in MeOH) and aq diammonium hydrogen citrate (80 mg/mL) as a matrix premixed just before loading the samples onto a plate. Thermal denaturation experiments were performed on a Perkin–Elmer Lambda 40 UV/vis

Spectrometer with PTP 6 (Peltier Temperature Programmer) in a buffer containing 100 mM NaCl, 10 mM Na-phosphate, 0.1 mM EDTA, pH 7.0.

4.1.1. (2*R*)-*N*-(2-Aminoethyl)-2,4-dihydroxybutyramide (2a). A solution of (*R*)-(+)-α-hydroxy-γ-butyrolactone (1.02 g, 10 mmol) in ethylenediamine (3.5 mL, 50 mmol) was kept at 55 °C overnight, evaporated, co-evaporated with toluene (30 mL) and *N*,*N*-diisopropylethylamine (1.8 mL, 10 mmol). The residue was triturated in Et_2O under argon and dried in vacuo to yield amide **2a** (1.63 g, 100%) as a white solid. ESI-TOF HRMS: *m*/*z*=163.1074 $[M+H]^+$, calcd for $[C_6H_{14}N_2O_3+H]^+$ 163.1077. ^1H NMR (DMSO-d_6) δ 7.81 (br s, 0.19H), 7.67 (br s, 0.81H) (CON*H*, exchangeable with D_2O), 3.93 (dd, 1H, J_1=3.6 Hz, J_2=8.6 Hz, C*H*O), 3.49 (m, 2H, CH_2O), 3.40–3.00 (br, 2H,‡ NH_2, exchangeable with D_2O), 3.07 (apparent q, 2H, *J*=6.3 Hz, CH_2NH), 2.57 (t, 2H, *J*=6.3 Hz, CH_2NH$_2$), 1.83 (m, 1H), 1.55 (m, 1H) (CH$_2$CH_2CH). ^{13}C NMR (DMSO-d_6) δ 174.63 (*C*=O), 69.07 (*C*HOH), 57.92 (*C*H$_2$OH), 41.97, 41.65 (*C*H$_2$*C*H$_2$), 38.07 (C*C*H$_2$C).

4.1.2. (2*R*)-*N*-(2-Aminoethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (2b). Compound **2b** was prepared in a similar manner as **2a** from (*R*)-(−)-pantolactone (3.25 g, 25 mmol) and ethylenediamine (9 mL, 125 mmol). After co-evaporation with toluene (100 mL) and *N*,*N*-diisopropylethylamine (4.4 mL, 25 mmol), trituration in Et_2O and drying, the desired amide **2b** (4.76 g, 100%) was obtained as a white solid. ESI-TOF HRMS: *m*/*z*=191.1388 $[M+H]^+$, calcd for $[C_8H_{18}N_2O_3+H]^+$ 191.1390. IR (KBr): 3342 cm^{-1}, ν (OH, NH); 2963 cm^{-1}, ν (sp^3-CH); 1654 cm^{-1}, ν (C=O); 1540 cm^{-1}, ν (NH). ^1H NMR (DMSO-d_6) δ 7.81 (br s, 0.08H), 7.67 (br s, 0.92H) (CON*H*, exchangeable with D_2O), 3.71 (s, 1H, C*H*O), 3.29 (d, 1H), 3.18 (d, 1H) (^2J=10.4 Hz, CH_2O), 3.20–2.80 (br, 2H, NH_2, exchangeable with D_2O), 3.07 (m, 2H, J_{HCCH}=J_{HNCH}=6.4 Hz, ^2J=12.8 Hz, CH_2NH), 2.57 (m, 2H, J_{HCCH}=6.4 Hz, ^2J=12.6 Hz, CH_2NH$_2$), 0.81 (s, 3H), 0.80 (s, 3H) (C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ 173.84 (*C*=O), 75.20 (*C*HOH), 66.18 (*C*H$_2$OH), 42.12, 41.53 (*C*H$_2$*C*H$_2$), 38.39 (C*C*Me$_2$C), 21.42, 20.81 (*C*H$_3$).

4.1.3. (2*R*)-*N*-Methyl-*N*-(2-methylaminoethyl)-2,4-dihydroxybutyramide (2c). A solution of (*R*)-(+)-α-hydroxy-γ-butyrolactone (1.02 g, 10 mmol) in *N*,*N*′-dimethylethylenediamine (5.2 mL, 50 mmol) was kept at 55 °C for 7 days, then evaporated, co-evaporated with *N*,*N*-diisopropylethylamine (1.8 mL, 10 mmol) and then toluene (3×30 mL) to give diol **2c** as a yellowish viscous oil (1.90 g, quant.). ESI-TOF HRMS: *m*/*z*=191.1381 $[M+H]^+$, calcd for $[C_8H_{18}N_2O_3+H]^+$ 191.1390. The compound was used without further purification in the next steps.

4.1.4. (2*R*)-*N*-[2-(9-Fluorenylmethoxycarbonylamino)-ethyl]-2,4-dihydroxybutyramide (3a). To a stirred solution of crude amine **2a** (1.63 g, 10 mmol) in 50% aq MeCN (20 mL), a solution of FmocOSu (3.38 g, 10 mmol) in MeCN (20 mL) was added in one portion and stirring continued overnight at ambient temperature. The precipitate formed was filtered off, washed with MeCN (10 mL), Et_2O (30 mL) and dried in vacuo to yield diol **3a** (2.50 g, 65%) as a white crystalline powder. An additional amount of product (0.90 g) was obtained by evaporation of combined filtrate, dissolving the residue in EtOAc (100 mL), washing with water (2×70 mL), drying (Na_2SO_4), evaporation and crystallisation from ^{i}PrOH. Total yield 3.40 g (88%), mp 133.5–134.0 °C (^{i}PrOH). R_f 0.13 ($CHCl_3$–MeOH 9:1 v/v). ESI-TOF HRMS: *m*/*z*=385.1759 $[M+H]^+$, calcd for $[C_{21}H_{24}N_2O_5+H]^+$ 385.1758. IR (KBr): 3321 cm^{-1}, ν (OH, NH); 2944 cm^{-1}, ν (sp^3-CH); 1694 cm^{-1}, ν (C=O); 1536 cm^{-1}, ν (NH); 1451 cm^{-1}, ν (sp^3-CH). ^1H NMR (DMSO-d_6) δ 7.88 (m, 2H, Ar*H* (H-4, H-5)), 7.77 (br s, 1H, CHCON*H*), 7.68 (m, 2H, Ar*H* (H-1, H-8)), 7.40 (m, 2H, Ar*H* (H-3, H-6)), 7.33 (m, 2H, Ar*H* (H-2, H-7)), 7.28 (br s, 1H, OCON*H*), 5.40 (d, 1H, *J*=5.4 Hz, CHO*H*), 4.40 (t, 1H, *J*=5.1 Hz, CH$_2$O*H*) (both exchangeable with D_2O), 4.30 (m, 2H, CH_2OCO), 4.21 (t, 1H, *J*=6.9 Hz, fluorene H-9), 3.94 (m, 1H, C*H*O) (in the presence of D_2O gives dd, J_1=3.7 Hz, J_2=8.4 Hz), 3.49 (m, 2H, CH_2OH), 3.16 (m, 2H), 3.07 (m, 2H) (NCH_2CH_2), 1.83 (m, 1H), 1.55 (m, 1H) (CH$_2$CH_2CH). ^{13}C NMR (DMSO-d_6) δ 174.91 (HN*C*=O), 156.64 (O*C*=O), 144.35 (2C), 141.18 (2C), 128.05 (2C), 127.51 (2C), 125.57 (2C), 120.55 (2C) (Ar, Fmoc), 69.04 (*C*HOH), 65.81 (*C*H$_2$OCO), 57.91 (*C*H$_2$OH), 47.18 (fluorene C-9), ~40‡ N*C*H$_2$), 38.66 (N*C*H$_2$), 38.04 (C*C*H$_2$C).

4.1.5. (2*R*)-*N*-[2-(9-Fluorenylmethoxycarbonylamino)-ethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (3b). Compound **3b** was prepared by treatment of amine **2b** (1.90 g, 10 mmol) in 50% aq MeCN (20 mL) by a solution of FmocOSu (3.38 g, 10 mmol) in MeCN (20 mL) at room temperature for 48 h. The mixture was filtered, evaporated, the residue was dissolved in EtOAc (200 mL), washed with water (150 mL), 5% $NaHCO_3$ (2×150 mL), then dried (Na_2SO_4), evaporated, co-evaporated with toluene (3×30 mL) and chromatographed on silica gel column using stepwise gradient elution with 0.5→1→2→3→5→8% MeOH in $CHCl_3$ (v/v). Fractions containing product were combined, evaporated and the residue was dried in vacuo to afford pure **3b** as a white solid (3.10 g, 75%), mp 64.0–65.0 °C. R_f 0.17 ($CHCl_3$–MeOH 9:1 v/v). ESI-TOF HRMS: *m*/*z*=435.1909 $[M+Na]^+$, calcd for $[C_{23}H_{28}N_2O_5+Na]^+$ 435.1890. IR (KBr): 3329 cm^{-1}, ν (OH, NH); 2957 cm^{-1}, ν (sp^3-CH); 1697 cm^{-1}, ν (C=O); 1537 cm^{-1}, ν (NH); 1450 cm^{-1}, ν (sp^3-CH bending). ^1H NMR (DMSO-d_6) δ 7.88 (d, 2H, *J*=7.3 Hz, Ar*H* (H-4, H-5)), 7.74 (br s, 1H, CHCON*H*, exchangeable with D_2O), 7.67 (d, 2H, *J*=7.5 Hz, Ar*H* (H-1, H-8)), 7.41 (m, 2H, Ar*H* (H-3, H-6)), 7.33 (m, 2H, Ar*H* (H-2, H-7)), 7.25 (br s, 1H, OCON*H*, exchangeable with D_2O), 5.33 (d, 1H, *J*=5.5 Hz, CHO*H*), 4.44 (t, 1H, *J*=5.5 Hz, CH$_2$O*H*) (both exchangeable with D_2O), 4.29 (m, 2H, CH_2OCO), 4.20 (m, 1H, fluorene H-9), 3.71 (d, 1H, *J*=5.5 Hz, C*H*O), 3.29 (m, 1H,‡ CH*H*OH), 3.25–3.03 (m, 5H, NCH_2CH_2, CH*H*OH), 0.80 (s, 3H), 0.78 (s, 3H) (C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ 173.66 (HN*C*=O), 156.63 (O*C*=O), 144.35 (2C), 141.18 (2C), 128.05 (2C), 127.50 (2C), 125.56 (2C), 120.55 (2C) (Ar, Fmoc), 75.63 (*C*HOH), 68.48 (*C*H$_2$OCO), 65.80 (*C*H$_2$OH), 47.17 (fluorene C-9), ~40 (2C,‡ N*C*H$_2$*C*H$_2$), 38.53 (C*C*Me$_2$C), 21.40, 20.79 (*C*H$_3$).

4.1.6. (2*R*)-*N*-Methyl-*N*-(2-[methyl(9-fluorenylmethoxycarbonyl)amino]ethyl)-2,4-dihydroxybutyramide (3c). The crude amine **2b** (1.14 g, 6 mmol) in 50% aq MeCN

‡ Calculated value; the signal of water or a solvent is also present in the region.

(15 mL) was treated with a solution of FmocOSu (2.03 g, 6 mmol) in MeCN (10 mL) for 48 h. The workup was similar to the above procedure. After column chromatography in 0.5→1→1.5→2→2.5% MeOH in $CHCl_3$ (v/v) the desired compound **3c** was isolated as a white foam (1.58 g, 64%), R_f 0.36 ($CHCl_3$–MeOH 9:1 v/v). ESI-TOF HRMS: *m/z*= 435.1878 $[M+Na]^+$, calcd for $[C_{23}H_{28}N_2O_5+Na]^+$ 435.1890. ^1H NMR (MeCN-d_3) δ 7.85 (d, 2H, *J*=7.3 Hz, Ar*H*, H-4, H-5), 7.79 (d, 0.57H, *J*=7.5 Hz, Ar*H*), 7.65 (d, 1.43H, *J*=7.3 Hz, Ar*H*) (H-1, H-8), 7.43 (m, 2H, Ar*H*, H-3, H-6), 7.36 (m, 2H, Ar*H*, H-2, H-7), 4.59–4.25 (m, 4H, C*H*O, C*H*CH_2OCO), 3.85–2.33 (m, 12H, CH_2OH, N(CH_3)CH_2CH_2NCH_3), 1.89–1.26 (m, 2H, CH_2CH_2CH). ^{13}C NMR (MeCN-d_3) δ 172.24 (MeN*C*=O), 155.80 (O*C*=O), 144.16 (2C), 141.09 (2C), 128.01 (2C), 127.35 (2C), 125.46 (2C), 120.47 (2C) (Ar, Fmoc), 68.88 (*C*HOH), 68.42 (*C*H_2OCO), 65.74 (*C*H_2OH), 47.10 (fluorene C-9), 39.55, 39.16 (N*C*H_2*C*H_2), 38.44 (C*C*H_2C), 35.10, 34.52 (*C*H_3).

4.1.7. (2*R*)-*O*^{4}-(4,4′-Dimethoxytrityl)-*N*-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-2,4-dihydroxybutyramide (4a). Diol **3a** (2.50 g, 6.5 mmol) was co-evaporated with pyridine (3×20 mL), dissolved in dry pyridine (50 mL), cooled in an ice bath, and DmtCl (2.40 g, 7.1 mmol) was added in three portions within 1 h. After disappearance of the starting material, the excess of DmtCl was quenched with MeOH (1 mL), and after 10 min the mixture was diluted with $CHCl_3$ (300 mL), washed with 2.5% $NaHCO_3$ (3×200 mL), water (2×200 mL), 20% NaCl (200 mL), then dried (Na_2SO_4), evaporated, co-evaporated with toluene (3×30 mL) and the residue was chromatographed on silica gel column using stepwise gradient $CHCl_3$–toluene 1:2→1:1→2:1→$CHCl_3$+1% Py (v/v/v). Fractions containing the product were combined, evaporated and the residue was dried in vacuo to afford **4a** as a white foam (3.6 g, 81%), R_f 0.16 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=709.2869 $[M+Na]^+$, calcd for $[C_{42}H_{42}N_2O_7+Na]^+$ 709.2884. ^1H NMR (DMSO-d_6) δ 7.88 (d, 2H, *J*=7.5 Hz, Ar*H* (fluorene H-4, H-5)), 7.78 (br s, 1H CHCON*H*), 7.67 (d, *J*=7.3 Hz, Ar*H*, fluorene, H-1, H-8), 7.41–7.12 (m, 14H, Ar*H* (Dmt+fluorene H-2, H-3, H-6, H-7), OCON*H*), 6.86 (d, 4H, *J*=8.9 Hz, Ar*H* (Dmt)), 5.37 (d, 1H, *J*=5.3 Hz, CHO*H*, exchangeable with D_2O), 4.29 (d, 2H, *J*=6.7 Hz, CH_2OCO), 4.18 (t, 1H, *J*=6.7 Hz, fluorene H-9), 3.96 (m, 1H, C*H*O) (in the presence of D_2O gives dd J_1=3.5 Hz, J_2=8.4 Hz), 3.72 (s, 6H, OCH_3), 3.16–2.98 (m, 6H, NCH_2CH_2, CH_2ODmt), 1.98 (m, 1H), 1.70 (m, 1H) (CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 174.17, 174.11 (HN*C*=O), 158.02, 158.00, 156.20 (O*C*=O), 149.69, 149.66, 149.64, 145.31, 143.95, 142.63, 140.79, 139.47, 137.48, 136.16, 136.11, 129.67, 128.97, 128.95, 128.25, 127.79, 127.73, 127.65, 127.34, 127.31, 127.11, 126.57, 125.36, 125.17, 123.94, 123.92, 121.42, 121.40, 120.15, 120.07, 120.05, 113.15, 109.76, 85.35, 68.65, 59.77, 55.05 (OMe), 46.79, 34.74, 21.08.

4.1.8. (2*R*)-*O*^{4}-(4,4′-Dimethoxytrityl)-*N*-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-2,4-dihydroxy-3,3-dimethylbutyramide (4b). Diol **3b** (2.10 g, 5 mmol) was tritylated in a similar manner with DmtCl (1.90 g, 5.5 mmol). Yield 3.50 g (97%), white foam, R_f 0.22 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=737.3205 $[M+Na]^+$, calcd for $[C_{44}H_{46}N_2O_7+Na]^+$ 737.3197. ^1H NMR (DMSO-d_6) δ 7.88 (d, 2H, *J*=7.3 Hz, Ar*H* (fluorene H-4, H-5)), 7.71 (br s, 1H, CHCON*H*), 7.66 (d, 2H, *J*=7.3 Hz, Ar*H* (fluorene H-1, H-8), 7.43–7.16 (m, 14H, Ar*H* (fluorene H-2, H-3, H-6, H-7, Dmt), OCON*H*), 6.86 (d, 4H, *J*=8.9 Hz, Ar*H* (Dmt)), 5.36 (d, 1H, *J*= 5.3 Hz, O*H*, exchangeable with D_2O), 4.28 (m, 2H, CH_2OCO), 4.21 (m, 1H, fluorene H-9), 3.80 (d, 1H, *J*= 5.3 Hz, C*H*O), 3.72 (s, 6H, OCH_3), 3.17–2.98 (m, 5H, NCH_2CH_2, CH*H*ODmt), 2.75 (d, 1H, ^2J=8.3 Hz, CH*H*ODmt), 0.92 (s, 3H), 0.78 (s, 3H) (C(CH_3)$_2$). ^{13}C NMR (DMSO-d_6) δ 172.73 (HN*C*=O), 157.97, 157.95, 157.93, 156.22 (O*C*=O), 149.66, 145.49, 143.94, 140.78, 136.21, 136.16, 136.11, 129.87, 129.83, 128.96, 128.94, 128.24, 127.88, 127.69, 127.65, 127.33, 127.09, 126.50, 125.36, 125.15, 123.94, 121.42, 120.16, 113.03, 84.95, 75.27, 68.36, 65.40, 55.05, 55.03, 55.00 (OMe), 46.78, 38.60, 38.12, 22.21, 21.08, 20.51.

4.1.9. (2*R*)-*O*^{4}-(4,4′-Dimethoxytrityl)-*N*-methyl-*N*-(2-[methyl-*N*′-(9-fluorenylmethoxycarbonyl)amino]-ethyl)-2,4-dihydroxybutyramide (4c). Compound **4c** was prepared from **3c** (1.48 g, 3.6 mmol) and DmtCl (1.37 g, 3.95 mmol). The product was purified by column chromatography in $CHCl_3$–toluene→1:4→1:1→2:1→$CHCl_3$+1% Py (v/v/v). Yield 2.28 g (89%), white foam, R_f 0.27 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=737.3202 $[M+Na]^+$, calcd for $[C_{44}H_{46}N_2O_7+Na]^+$ 737.3197. ^1H NMR (DMSO-d_6) δ 7.87 (m, 2H, Ar*H* (fluorene H-4, H-5)), 7.58 (m, 2H, Ar*H* (fluorene H-1, H-8)), 7.42–7.12 (m, 13H, Ar*H* (fluorene H-2, H-3, H-6, H-7, Dmt)), 6.84 (m, 4H, Ar*H* (Dmt)), 4.79 (m, 1H, O*H*, exchangeable with D_2O), 4.63–4.20 (m, 4H, C*H*O, C*H*CH_2OCO), 3.72 (s, 6H, OCH_3), 3.55–2.55 (m, 12H,‡ N(CH_3)CH_2CH_2NCH_3, CH_2ODmt), 1.83–1.43 (m, 2H, CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 173.00, 172.93 (MeN*C*=O), 158.03, 157.68 (O*C*=O), 143.97, 143.90, 143.85, 142.63, 140.81, 139.47, 137.48, 136.06, 136.00, 129.65, 128.96, 128.94, 128.24, 127.80, 127.70, 127.65, 127.33, 127.12, 126.61, 125.35, 124.95, 121.41, 120.11, 120.06, 120.04, 113.15, 113.11, 109.76, 85.46, 65.06, 64.98, 59.87, 55.06, 55.05, 55.02 (OMe), 34.45, 21.08.

4.1.10. (2*R*)-*O*^{4}-(4,4′-Dimethoxytrityl)-*N*-methyl-*N*-(2-[*N*′-methyl-*N*′-trifluoroacetylamino]ethyl)-2,4-dihydroxybutyramide (4d). The crude diol **2c** (0.75 g, 4 mmol) was co-evaporated with dry MeOH (20 mL), dissolved in the mixture of MeOH (10 mL), Et_3N (0.6 mL, 4 mmol) and CF_3CO_2Me (2.0 mL 10 mmol). The mixture was stirred at ambient temperature for 30 min and evaporated to give crude **3d**. This was co-evaporated with pyridine and tritylated with DmtCl (2.02 g, 6.0 mmol) as above. The product **4d** was chromatographed on silica gel eluting with $CHCl_3$–toluene 1:2→1:1→$CHCl_3$+0.25% Et_3N (v/v/v). Yield 1.44 g (93%) as a white foam, R_f 0.23 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=611.2331 $[M+Na]^+$, calcd for $[C_{31}H_{35}F_3N_2O_6+Na]^+$ 611.2339. ^1H NMR (DMSO-d_6) δ 7.38–7.21 (m, 9H, Ar*H* (Dmt)), 6.87 (d, 4H, *J*=8.7 Hz, Ar*H* (Dmt)), 5.07 (d, 0.04H, *J*=7.8 Hz), 4.91 (d, 0.16H, *J*=7.8 Hz), 4.75 (d, 0.19H, *J*=7.8 Hz), 4.59 (d, 0.61H, *J*=8.0 Hz) (O*H*, exchangeable with D_2O), 4.42 (m, 1H, C*H*O), 3.73 (s, 6H, OCH_3), 3.80–3.26 (m, 4H,‡ CH_2O, CH_2NTfa), 3.15–2.78 (m, 8H, N(CH_3)CH_2CH_2N(CH_3)Tfa),

1.86–1.50 (m, 2H, CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 173.90, 173.58, 172.96 (MeNC=O), 158.05, 156.13, 155.85, 155.57, 145.24, 145.18, 136.03, 129.62, 128.93, 128.24, 127.81, 127.77, 127.72, 127.66, 126.62, 125.35, 117.49, 115.18, 113.17, 113.13, 85.46, 65.13, 64.98, 59.86, 55.05 (OMe), 45.94, 45.76, 43.76, 40.19, 40.02, 39.86, 35.39, 35.03, 34.85, 34.82, 34.80, 34.53, 34.44, 34.36, 33.46.

4.1.11. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-2,4-dihydroxybutyramide (5a). Compound **4a** (1.38 g, 2 mmol) was co-evaporated with dry DCM (2×20 mL), dissolved in dry DCM, diisopropylammonium tetrazolide (0.51 g, 3 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (1 mL, 3 mmol) were added, and the mixture was stirred under argon for 2 h. After conversion of the starting compound is complete (monitoring by TLC, $CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v) the mixture was diluted with EtOAc and washed with 5% $NaHCO_3$ (2×100 mL) and 20% NaCl (100 mL). The organic layer was dried over Na_2SO_4, evaporated to dryness, and the residue was dissolved in 30 mL DCM and precipitated into cold (−10 °C) hexane (500 mL), the solid was filtered off, co-evaporated with dry DCM (3×20 mL) and dried in vacuo to afford phosphoramidite **5a** (1.51 g, 85%) as a white foam, R_f 0.34, 0.43 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m*/*z*=909.4066 $[M+Na]^+$, calcd for $[C_{51}H_{59}N_4O_8P+Na]^+$ 909.3963. ^{31}P NMR (MeCN-d_3) δ 151.13, 148.73 (diastereomers, ∼1:2). ^1H NMR (MeCN-d_3) δ 7.85–7.19 (m, 18H, Ar*H* (fluorene, Dmt), CCON*H*), 6.94 (m, 1H, OCON*H*), 6.84 (m, 4H, Ar*H* (Dmt)), 4.35–4.01 (m, 4H, C*H*CH_2OCO, C*H*O), 3.76 (m, 6H, OCH_3), 3.65–2.48 (m, 12H, NCH_2CH_2, $POCH_2CH_2$, NC*H*, CH_2ODmt), 2.18–2.00 (m, 2H, CH_2CH_2CH), 1.30–0.96 (m, 12H, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 173.57, 173.27, 173.16 (HNC=O), 159.49, 157.49 (OC=O), 146.37, 145.14, 142.08, 137.31, 130.86, 128.96, 128.67, 128.63, 128.06, 127.60, 126.34, 126.10, 120.91, 113.91, 86.88, 86.77, 72.09, 66.98, 60.55, 60.26, 59.74, 59.59, 59.12, 58.07, 55.81 (OMe), 55.24, 48.09, 47.19, 45.96, 43.99, 43.89, 41.49, 39.73, 35.29, 34.84, 24.87, 23.12, 23.05, 21.91, 20.86, 20.51, 19.99.

4.1.12. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-2,4-dihydroxy-3,3-dimethylbutyramide (5b). Compound **4b** (1.87 g, 2.6 mmol) was phosphitylated as above with diisopropylammonium tetrazolide (0.67 g, 3.9 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (1.27 mL, 3.9 mmol) to afford **5b** (1.60 g, 67%) as a white foam. ESI-TOF HRMS: *m*/*z*=937.4284 $[M+Na]^+$, calcd for $[C_{53}H_{63}N_4O_8P+Na]^+$ 937.4276. ^{31}P NMR (MeCN-d_3) δ 152.38, 149.04 (diastereomers, ∼1:2). ^1H NMR (MeCN-d_3) δ 7.84 (d, 2H, *J*=7.4 Hz, Ar*H* (fluorene H-4, H-5)), 7.66 (m, 2H, Ar*H* (fluorene H-1, H-8)), 7.48–7.22 (m, 15H, Ar*H* (fluorene H-2, H-3, H-6, H-7, Dmt), CHCON*H*, OCON*H*), 6.86 (m, 4H, Ar*H* (Dmt)), 4.37–4.31 (m, 2H, CH_2OCO), 4.23 (m, 1H, fluorene H-9), 4.03–3.92 (m, 1H, C*H*O), 3.77 (c, 6H, OCH_3), 3.73–2.70 (m, 10H, NCH_2CH_2, $POCH_2$, NC*H*, CH_2ODmt), 2.46 (t, 0.8H, *J*=6.0 Hz), 2.29 (t, 1.2H, *J*=6.0 Hz) (CH_2CN, diastereomers), 1.28–0.95 (m, 18H, $CH_2C(CH_3)_2CH$, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 171.71 (HNC=O), 159.46, 157.51 (OC=O), 146.53, 145.17, 142.08, 137.23, 131.21, 131.13, 129.19, 129.08, 128.61, 128.05, 127.55, 126.05, 120.91, 119.41, 113.78, 86.54, 78.96, 69.24, 68.85, 66.99, 66.87, 59.79, 59.64, 59.06, 55.81 (OMe), 55.24, 48.06, 47.27, 45.91, 44.18, 44.04, 43.94, 41.48, 40.06, 39.78, 25.03, 24.83, 23.05, 22.83, 22.24, 21.41, 20.87, 20.19.

4.1.13. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-methyl-*N*-(2-[methyl(trifluoroacetyl)amino]ethyl)-2,4-dihydroxybutyramide (5c). Compound **4d** (1.18 g, 2.0 mmol) was phosphitylated with diisopropylammonium tetrazolide (510 mg, 3.0 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (1.0 mL, 3 mmol) under argon for 2 h. The conversion of the starting material was monitored by TLC ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). The compound was precipitated from DCM (30 mL) into cold hexane. Yield 1.18 g (77%), white foam, R_f 0.29, 0.39 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m*/*z*=811.3442 $[M+Na]^+$, calcd for $[C_{40}H_{52}F_3N_4O_7P+Na]^+$ 811.3418. ^{31}P NMR (MeCN-d_3) δ 150.42 (0.19P), 150.03 (0.36P), 149.86 (0.11P), 149.30 (0.34P). ^1H NMR (MeCN-d_3) δ 7.49–7.20 (m, 9H, Ar*H* (Dmt)), 6.89 (m, 4H, Ar*H* (Dmt)), 4.88–4.64 (m, 0.44H), 4.20–4.02 (m, 0.56H) (C*H*O), 3.79 (m, 6H, OCH_3), 3.71–2.48 (m, 18H, $POCH_2CH_2$, NC*H*, CH_2ODmt, $CH_3NCH_2CH_2NCH_3$), 2.14–1.81 (m, 2H,‡ CH_2CH_2CH), 1.29–1.03 (m, 12H, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 172.59 (MeNC=O), 159.57, 146.41, 146.11, 137.23, 130.85, 128.87, 128.72, 127.68, 119.49, 113.95, 87.04, 86.88, 69.15, 60.88, 60.49, 59.61, 59.45, 59.28, 59.12, 55.83, 55.24 (OMe), 47.18, 46.72, 45.97, 45.22, 43.95, 43.85, 35.83, 35.68, 34.95, 24.90, 24.80, 24.74, 23.13, 23.06, 20.88.

4.1.14. (2*R*)-*N*-[2-(Imidazol-4-yl)ethyl]-2,4-dihydroxybutyramide (7a). A solution of (*R*)-(+)-α-hydroxy-γ-butyrolactone (1.02 g, 10 mmol) and histamine (1.17 g, 10 mmol) in EtOH (10 mL) was kept at 55 °C for 24 h. The solid formed was filtered off, washed with EtOH (5 mL), Et_2O (2×5 mL) and dried in vacuo to afford **7a** (1.77 g, 83%) as white crystals, mp 149.0–149.5 °C (EtOH). ESI-TOF HRMS: *m*/*z*=214.1187 $[M+H]^+$, calcd for $[C_9H_{15}N_3O_3+H]^+$ 214.1186. IR (KBr): 3392 cm^{-1}, ν (OH); 3164 cm^{-1}, ν (NH); 2904 cm^{-1}, ν (sp^3-CH); 1644 cm^{-1}, ν (C=O); 1533 cm^{-1}, ν (NH). ^1H NMR (CD_3OD) δ 7.61 (m, 1H, imidazole H-2), 6.89 (br s, 1H, imidazole H-5), 4.14 (dd, 1H, J_1=3.7 Hz, J_2=8.6 Hz, C*H*O), 3.71 (m, 2H, CH_2O), 3.51 (t, 2H, *J*=7.0 Hz, NCH_2), 2.82 (t, 2H, *J*=7.0 Hz, NCH_2CH_2), 2.01 (m, 1H), 1.74 (m, 1H) (CH_2CH_2CH). ^{13}C NMR (CD_3OD) δ 177.21 (*C*O), 136.13 (3C, imidazole C-2, C-4, C-5), 70.56 (*C*HOH), 59.57 (CH_2OH), 39.88, 38.23 (NCH_2CH_2), 27.80 (CH_2CH_2CH).

4.1.15. (2*R*)-*N*-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxybutyramide (8a). To a stirred solution of diol **7a** (0.745 g, 3.5 mmol) in 50% aq dioxan (50 mL), Boc_2O (0.839 g, 3.8 mmol) was added at room temperature and stirring was continued for 5 h. After completion of the reaction (monitored by TLC, $CHCl_3$–MeOH 4:1 v/v), the solvent was evaporated, and the residue was chromatographed on a silica gel column using stepwise gradient elution with 0→2→4→6→8% MeOH in $CHCl_3$. Yield 0.83 g (76%), white crystals, mp 92.0–93.0 °C. R_f 0.37

($CHCl_3$–MeOH 5:1 v/v). ESI-TOF HRMS: m/z=314.1715 $[M+H]^+$, calcd for $[C_{14}H_{23}N_3O_5+H]^+$ 314.1710. IR (KBr): 3428 cm^{-1}, ν (OH); 3316 cm^{-1}, 3103 cm^{-1}, ν (NH); 2938 cm^{-1}, ν (sp^3-CH); 1653 cm^{-1}, ν (C=O); 1536 cm^{-1}, ν (NH). ^1H NMR (DMSO-d_6) δ 8.10 (br s, 1H, imidazole H-2), 7.79 (m, 1H, CON*H*, exchangeable with D_2O), 7.28 (br s, 1H, imidazole H-5), 5.41 (d, 1H, J=5.5 Hz, CHO*H*), 4.39 (t, 1H, J=5.3 Hz, CH_2O*H*) (both exchangeable with D_2O), 3.91 (m, 1H, C*H*O), 3.48 (m, 2H, CH_2O), 3.33 (m, 2H,‡ NCH_2), 2.63 (t, 2H, J=7.0 Hz, NCH_2CH_2), 1.81 (m, 1H, CH_2CH*H*CH), 1.52 (m, 10H, CH_3 (Boc), CH_2CH*H*CH). ^{13}C NMR (DMSO-d_6) δ 174.53 (HN*C*=OC), 147.19 (N*C*OO), 141.45 (imidazole C-4), 137.14 (imidazole C-2), 113.86 (imidazole C-4), 85.48 ($C(CH_3)_3$), 69.01 (*C*HOH), 57.91 (CH_2OH), 37.97 (2C, NCH_2CH_2), 28.22 (CH_2CH_2CH), 27.85 (3C, CH_3).

4.1.16. (2*R*)-*N*-{2-[1-(*tert*-Butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (8b1) and (2*R*)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-5-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (8b2). A solution of (*R*)-(−)-pantolactone (4.3 g, 33 mmol) and histamine (3.3 g, 30 mmol) in abs EtOH (20 mL) was kept at 55 °C for 72 h, then evaporated, co-evaporated with toluene to afford (2*R*)-*N*-[2-(imidazol-4-yl)ethyl]-2,4-dihydroxy-3,3-dimethylbutyramide **7b** as a yellow oil. The product was used without further purification. ESI-TOF HRMS: m/z=242.1501 $[M+H]^+$, calcd for $[C_{11}H_{19}N_3O_3+H]^+$ 242.1499. The crude **7b** was treated with Boc_2O (7.20 g, 33 mmol) in 50% aq dioxane (50 mL) as above to afford two isomers, **8b1** (3.88 g, 38%) and **8b2** (1.75 g, 17%) as white solids, R_f 0.54 and 0.48, correspondingly ($CHCl_3$–MeOH 4:1 v/v). 'Fast moving' product **8b1**: ESI-TOF HRMS: m/z=342.2019 $[M+H]^+$, calcd for $[C_{16}H_{27}N_3O_5+H]^+$ 342.2023. ^1H NMR (DMSO-d_6) δ 8.10 (br s, 1H, imidazole H-2), 7.75 (m, 1H, CON*H*, exchangeable with D_2O), 7.29 (br s, 1H, imidazole H-5), 5.31 (d, 1H, J=5.5 Hz, CHO*H*), 4.43 (t, 1H, J=5.4 Hz, CH_2O*H*) (both exchangeable with D_2O), 3.69 (d, 1H, J=5.5 Hz, C*H*O), 3.44–3.14 (m, 4H, CH_2O (^2J=10.3 Hz, J_{HCOH}=5.4 Hz), NCH_2), 2.64 (m, 2H, NCH_2CH_2), 1.55 (s, 9H, CH_3 (Boc)), 0.77 (s, 3H), 0.75 (s, 3H) ($C(CH_3)_2$). ^{13}C NMR (DMSO-d_6) δ 173.30 (HN*C*=OC), 147.19 (N*C*OO), 141.47 (imidazole C-4), 137.13 (imidazole C-2), 113.94 (imidazole C-4), 85.43 ($C(CH_3)_3$), 75.58 (*C*HOH), 68.52 (CH_2OH), ~40,‡ 37.97 (NCH_2CH_2), 28.23 (CH_2CCH), 27.85 (3C, CH_3), 21.29, 20.75 ($C(CH_3)_2$). 'Slow moving' product **8b2**: ESI-TOF HRMS: m/z= 342.2013 $[M+H]^+$, calcd for $[C_{16}H_{27}N_3O_5+H]^+$ 342.2023. The compound purified by column chromatography was kept at 4 °C for one month before recording its NMR spectrum. NMR (as well as TLC) showed considerable $\pi\rightarrow\tau$ Boc migration (conversion >50%). ^1H NMR (DMSO-d_6) of 'slow moving' product **8b2** (deduced from NMR spectrum of the mixture of **8b1** and **8b2**) δ 8.08 (br s, 1H, imidazole H-2), 7.76 (m, 1H, CON*H*, exchangeable with D_2O), 6.79 (br s, 1H, imidazole H-4), 5.31 (d, 1H, J=5.5 Hz, CHO*H*), 4.44 (t, 1H, J=5.4 Hz, CH_2O*H*) (both exchangeable with D_2O), 3.70 (d, 1H, J=5.5 Hz, C*H*O), 3.45–3.13 (m, 4H,‡ CH_2O, NCH_2), 2.91 (m, 2H, NCH_2CH_2), 1.57 (s, 9H, CH_3 (Boc)), 0.77 (s, 3H), 0.76 (s, 3H) ($C(CH_3)_2$).

4.1.17. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxybutyramide (9a). Diol **8a** (0.78 g, 2.5 mmol) was tritylated with DmtCl (0.93 g, 2.75 mmol) according to the procedure for **4a**. The product was purified by chromatography using a stepwise gradient $CHCl_3$–toluene 1:2→1:1→2:1→$CHCl_3$+ 0.25% Py (v/v/v), then 10%→50% EtOAc in $CHCl_3$+ 0.25% Py (v/v/v). Yield 1.31 g (86%), white foam, R_f 0.46 ($CHCl_3$–MeOH 20:1+1% Et_3N v/v/v). ESI-TOF HRMS: m/z=638.2766 $[M+Na]^+$, calcd for $[C_{35}H_{41}N_3O_7+Na]^+$ 638.2837. ^1H NMR (DMSO-d_6) δ 8.10 (br s, 1H, imidazole H-2), 7.80 (m, 1H, CON*H* (exchangeable with D_2O)), 7.37–7.22 (m, 10H, Ar*H* (Dmt), imidazole H-5), 6.87 (d, 4H, J=8.9 Hz, Ar*H* (Dmt)), 5.40 (d, 1H, J=5.7 Hz, CHO*H*, exchangeable with D_2O), 3.98 (m, 1H, C*H*O), 3.72 (s, 6H, OCH_3), 3.30 (m, 2H‡), 3.04 (m, 2H) (CH_2N, CH_2O), 2.58 (t, 2H, J=7.0 Hz, NCH_2CH_2), 1.96 (m, 3H), 1.69 (m, 3H) (CH_2CH_2CH), 1.55 (s, 9H, CH_3 (Boc)). ^{13}C NMR (DMSO-d_6) δ 173.76 (HN*C*=O), 158.01, 146.80, 145.29, 141.03, 136.74, 136.10, 136.07, 129.66, 128.94, 128.24, 127.78, 127.72, 126.56, 125.36, 113.42, 113.14, 85.35, 85.08, 68.60, 59.73, 55.05 (OMe), 37.60, 34.77, 27.81, 27.49, 27.48, 27.45, 27.43, 27.41 (CMe_3), 21.08.

4.1.18. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (9b1) and (2*R*)-O^4-(4,4′-dimethoxytrityl)-*N*-{2-[1-(*tert*-butyloxycarbonyl)imidazol-5-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (9b2). The 'fast moving' product **8b1** (3.7 g, 11 mmol) was tritylated with DmtCl (4.15 g, 12 mmol) as described above and isolated from silica gel column eluted with $CHCl_3$–toluene 1:2→1:1→2:1→$CHCl_3$+0.25% Py (v/v/v), then 2.5%→5% EtOAc in $CHCl_3$+0.25% Py (v/v/v). Fractions containing products were combined, evaporated and the residues were dried in vacuo to afford **9b1** (3.95 g, 55%) and **9b2** (2.60 g, 37%) as white foams, R_f 0.53 and 0.47, correspondingly ($CHCl_3$–MeOH 20:1+1% Et_3N v/v/v). 'Fast moving' product **9b1**: ESI-TOF HRMS: m/z=666.3163 $[M+Na]^+$, calcd for $[C_{37}H_{45}N_3O_7+Na]^+$ 666.3150. ^1H NMR (MeCN-d_3) δ 7.96 (m, 1H, imidazole H-2), 7.48–7.15 (m, 10H, Ar*H* (Dmt) imidazole H-5), 6.94 (br s, 1H, CON*H* (exchangeable with D_2O)), 6.86 (d, 4H, J=8.8 Hz, Ar*H* (Dmt)), 3.94 (d, 1H, J=5.7 Hz, O*H* (exchangeable with D_2O)), 3.78 (s, 6H, OCH_3), 3.67 (d, 1H, J=5.7 Hz, C*H*O), 3.37 (m, 2H, CH_2N), 3.07 (d, 1H, ^2J=8.7 Hz), 2.85 (d, 1H, ^2J=8.7 Hz) (CH_2O), 2.61 (m, 2H, NCH_2CH_2), 1.60 (s, 9H, CH_3 (Boc)), 0.96 (s, 3H), 0.88 (s, 3H) ($C(CH_3)_2$). ^{13}C NMR (MeCN-d_3) δ 172.96 (HN*C*=O), 158.47, 145.98, 145.46, 141.37, 136.89, 136.58, 136.39, 129.90, 128.78, 128.55, 127.83, 127.72, 126.91, 125.40, 113.88, 113.69, 86.55, 86.38, 68.85, 59.37, 55.03 (OMe), 38.43, 34.87, 28.01, 27.87, 27.63, 27.57, 27.39, 27.31 (CMe_3), 21.29, 21.18, 19.95. Compound **9b2** was stored at 4 °C for one month before recording its NMR spectrum. NMR and TLC showed complete conversion **9b2**→**9b1** during storage.

4.1.19. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxybutyramide (10a). Compound **9a** (1.23 g, 2.0 mmol) was phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.95 mL, 3 mmol) and diisopropylammonium tetrazolide (510 mg, 3.0 mmol) to afford phosphoramidite **10a** (1.29 g, 80%) as a white foam, R_f 0.37, 0.26 ($CHCl_3$–EtOAc

1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=838.3908 $[M+Na]^+$, calcd for $[C_{44}H_{58}N_5O_8P+Na]^+$ 838.3915. ^{31}P NMR (MeCN-d_3) δ 150.61, 148.64 (diastereomers, ~9:10). ^1H NMR (MeCN-d_3) δ 8.01 (br s, 1H, imidazole H-2), 7.46–7.17 (m, 10H, Ar*H* (Dmt), imidazole H-5), 7.07 (m, 0.59H), 7.01 (m, 0.41H) (CON*H*, diastereomers), 6.87 (m, 4H, Ar*H* (Dmt)), 4.31 (m, 0.41H), 4.24 (m, 0.59H) (C*H*O, diastereomers), 3.78 (s, 6H, OCH_3), 3.67–2.51 (m, 12H, CH_2O, NCH_2CH_2, $POCH_2CH_2$, NC*H*), 2.18–2.00 (m, 2H, CH_2CH_2CH), 1.61 (s, 9H, CH_3 (Boc)), 1.27–0.98 (m, 12H, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 170.23 (HN*C*=O), 158.87, 147.16, 145.97, 141.67, 137.12, 136.19, 130.27, 129.71, 128.47, 127.98, 127.36, 126.87, 119.99, 113.88, 113.11, 85.44, 77.77, 68.32, 59.12, 58.84, 55.14 (OMe), 44.88, 42.52, 42.35, 38.05, 37.86, 27.54 (CMe_3), 24.78, 24.17, 23.06, 22.24, 22.08, 19.97.

4.1.20. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-{2-[1-(*tert*-butoxycarbonyl)imidazol-4-yl]ethyl}-2,4-dihydroxy-3,3-dimethylbutyramide (10b). Compound **9b1** (3.22 g, 5.0 mmol) was phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (2.38 mL, 7.5 mmol) and diisopropylammonium tetrazolide (1.28 g, 7.5 mmol) to give compound **10b** as a white foam (2.36 g, 60%), R_f 0.38, 0.30 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=866.4238 $[M+Na]^+$, calcd for $[C_{46}H_{62}N_5O_8P+Na]^+$ 866.4228. ^{31}P NMR (MeCN-d_3) δ 152.29, 149.11 (diastereomers, ~9:10). ^1H NMR (MeCN-d_3) δ 7.97 (br s, 1H, imidazole H-2), 7.49–7.18 (m, 11H, Ar*H* (Dmt), imidazole H-5, CON*H*), 6.87 (m, 4H, Ar*H* (Dmt)), 3.97 (d, 0.55H, J_{HCOP}=12.9 Hz), 3.93 (d, 0.45H, J_{HCOP}=9.9 Hz) (C*H*O, diastereomers), 3.79 (m, 6H, OCH_3), 3.75–2.51 (m, 12H, CH_2O, NCH_2CH_2, $POCH_2CH_2$, NC*H*), 1.59 (s, 9H, CH_3 (Boc)), 1.27–0.94 (m, 18H, $C(CH_3)_2$, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 169.16 (HN*C*=O), 157.99, 146.76, 145.27, 140.91, 136.72, 135.88, 129.94, 129.81, 127.93, 127.80, 127.67, 126.54, 118.79, 113.58, 112.98, 85.08, 77.31, 67.91, 58.62, 58.23, 55.03 (OMe), 44.58, 42.82, 42.55, 37.76, 37.53, 27.43 (CMe_3), 24.27, 24.08, 22.66, 22.16, 21.96, 21.10, 20.67, 19.81, 19.42.

4.1.21. (2*R*)-*N*-(Pyren-1-ylmethyl)-2,4-dihydroxybutyramide (11a). A solution of (*R*)-(+)-α-hydroxy-γ-butyrolactone (1.02 g, 10 mmol) and 1-pyrenemethylamine (2 g, 9 mmol) in ethanol (5 mL) was kept at 55 °C for one week, evaporated and co-evaporated with toluene. The residue was triturated in EtOAc–toluene and dried in vacuo to yield amide **11a** (2.62 g, 88%) as white crystals, mp 162.0–164.0 °C (EtOAc–toluene). ESI-TOF HRMS: *m/z*=356.1260 $[M+Na]^+$, calcd for $[C_{21}H_{19}NO_3+Na]^+$ 356.1257. IR (KBr): 3330 cm^{-1}, ν (OH); 3273 cm^{-1}, ν (NH); 3040 cm^{-1}, ν (sp^2-CH); 1642 cm^{-1}, ν (C=O); 1537 cm^{-1}, ν (NH); 1044 cm^{-1}, ν (C–O); 839 cm^{-1}, ν (ArH). ^1H NMR (DMSO-d_6) δ 8.42 (m, 2H, Ar*H* (H-10), N*H*), 8.33–8.04 (m, 8H, Ar*H*), 5.52 (d, 1H, *J*=5.1 Hz, CHO*H*, exchangeable with D_2O), 5.05 (d, 2H, *J*=5.1 Hz, NCH_2), 4.43 (m, 1H, CH_2OH, exchangeable with D_2O), 4.07 (m, 1H, C*H*O) (in the presence of D_2O gives dd with J_1=3.1 Hz and J_2=7.1 Hz), 3.53 (m, 2H, CH_2O), 1.92 (m, 1H), 1.64 (m, 1H) (CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 174.68 (CO), 133.61, 131.25, 130.76, 130.44, 128.41, 127.90, 127.83, 127.39, 126.90, 126.65, 125.62, 125.56, 125.11, 124.44 (2C), 123.69 (pyrene), 69.19 (*C*HOH), 57.94 (CH_2OH), ~40‡ (NCH_2), 38.22 (CCH_2C).

4.1.22. (2*R*)-*N*-(Pyren-1-ylmethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (11b). A solution of (*R*)-(−)-pantolactone (3.25 g, 25 mmol) and 1-pyrenemethylamine (4.6 g, 20 mmol) in acetone (50 mL) was kept at 55 °C for 7 days, then evaporated and chromatographed on silica gel column using stepwise gradient $CHCl_3$–toluene 1:1→2:1→$CHCl_3$ (v/v), then 0.5%→1%→2% MeOH in $CHCl_3$ (v/v). Fractions containing the product were combined, evaporated and the residue was dried in vacuo to afford pure **11b** (6.64 g, 92%) as a yellow foam, R_f 0.24 ($CHCl_3$–MeOH 4:1 (v/v)). ESI-TOF HRMS: *m/z*=384.1577 $[M+Na]^+$, calcd for $[C_{23}H_{23}NO_3+Na]^+$ 384.1570. IR (KBr): 3396 cm^{-1}, ν (OH); 3041 cm^{-1}, ν (sp^2-CH); 1649 cm^{-1}, ν (C=O); 1529 cm^{-1}, ν (NH); 1041 cm^{-1}, ν (C–O); 841 cm^{-1}, ν (Ar-H). ^1H NMR (DMSO-d_6) δ 8.49 (d, 1H, *J*=8.1 Hz, Ar*H* (H-10)), 8.38 (m, 1H, N*H*, exchangeable with D_2O), 8.32–8.06 (m, 8H, Ar*H*), 5.43 (d, 1H, *J*=4.1 Hz, CHO*H*, exchangeable with D_2O), 5.04 (m, 2H, J_{HNCH}=5.1 Hz, ^2J=12.2 Hz, NCH_2), 4.45 (m, 1H, CH_2OH, exchangeable with D_2O), 3.85 (d, *J*=4.1 Hz, C*H*O), 3.30 (m, 1H‡), 3.20 (m, 1H) (CH_2O, in the presence of D_2O gives two d, ^2J=9.2 Hz), 0.82 (s, 3H), 0.79 (s, 3H) ($C(CH_3)_2$). ^{13}C NMR (DMSO-d_6) δ 173.38 (CO), 133.67, 131.24, 130.78, 130.47, 128.47, 127.83 (2C), 127.40, 126.84, 126.63, 125.60 (2C), 125.04, 124.45 (2C), 123.93 (pyrene), 75.71 (*C*HOH), 68.46 (CH_2OH), ~40‡ (NCH_2), 37.21 ($C(CH_3)_2$), 21.51, 20.83 (CH_3).

4.1.23. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-*N*-(pyren-1-ylmethyl)-2,4-dihydroxybutyramide (12a). Diol **11a** (1.29 g, 3.9 mmol) was tritylated with DmtCl (1.40 g, 4.1 mmol) as described for **4a**. The product was purified by chromatography in $CHCl_3$–toluene 1:21:1→2:1→$CHCl_3$+0.25% Py (v/v/v), then 10%→50% EtOAc in $CHCl_3$+0.25% Py (v/v/v). Yield 1.33 g (62%), white foam, R_f 0.28 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: *m/z*=658.2572 $[M+Na]^+$, calcd for $[C_{42}H_{37}NO_5+Na]^+$ 658.2564. ^1H NMR (DMSO-d_6) δ 8.42–7.99 (m, 10H, N*H*, Ar*H* (pyrene)), 7.36–7.13 (m, 9H, Ar*H* (Dmt)), 6.81 (m, 4H, Ar*H* (Dmt)), 5.50 (d, 1H, *J*=4.1 Hz, O*H*, exchangeable with D_2O), 5.01 (m, 2H, J_{HNCH}=5.1 Hz, ^2J=12.2 Hz, NCH_2), 4.12 (m, 1H, C*H*O) (in the presence of D_2O gives dd with J_1=3.1 Hz and J_2=6.1 Hz), 3.69 (s, 6*H*, CH_3), 3.07 (m, 2H, CH_2O), 2.04 (m, 1H), 1.82 (m, 1H) (CH_2CH_2CH). ^{13}C NMR (DMSO-d_6) δ 173.80 (CO), 157.98, 145.33, 137.39, 136.13, 136.07, 133.11, 130.85, 130.35, 130.05, 129.65, 128.94, 128.24, 128.03, 127.78, 127.69, 127.49, 127.43, 127.01, 126.60, 126.55, 126.25, 125.35, 125.24, 125.17, 124.68, 124.06, 124.01, 123.29, 113.10, 85.36, 68.73 (*C*HOH), 59.68, 55.01 (OMe), 34.88 (CCH_2C).

4.1.24. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-*N*-(pyren-1-ylmethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (12b). Diol **11b** (6.48 g, 18 mmol) was tritylated with DmtCl (6.80 g, 20 mmol) as above. The compound was chromatographed on a silica gel column eluted with 2.5%→5%→7.5%→10%→12.5%→15% EtOAc in toluene+0.25% Et_3N (v/v/v). Yield 10.0 g (83%), yellow foam, R_f 0.35

($CHCl_3$+1% Et_3N v/v/v). ESI-TOF HRMS: m/z=686.2872 $[M+Na]^+$, calcd for $[C_{44}H_{41}NO_5+Na]^+$ 686.2877. ^1H NMR (DMSO-d_6) δ 8.46–8.02 (m, 10H, N*H*, Ar*H* (pyrene)), 7.40–7.16 (m, 9H, ArH (Dmt)), 6.83 (d, 4H, J=7.1 Hz, Ar*H* (Dmt)), 5.46 (d, 1H, J=4.1 Hz, O*H*, exchangeable with D_2O), 4.99 (m, 2H, NCH_2), 3.92 (m, 1H, C*H*O), 3.70 (s, 6H, OCH_3), 3.01 (d, 1H, ^2J=7.1 Hz), 2.78 (d, 1H, ^2J=7.1 Hz) (CH_2O), 0.93 (s, 3H), 0.82 (s, 3H) ($C(CH_3)_2$). ^{13}C NMR (DMSO-d_6) δ 172.47 (CO), 157.93, 145.50, 136.19, 136.12, 133.25, 130.84, 130.36, 130.04, 129.85, 128.94, 128.24, 128.02, 127.85, 127.69, 127.42, 126.29, 126.89, 126.48, 126.23, 125.35, 125.21, 125.15, 124.63, 124.05, 124.01, 123.46, 113.01, 84.95, 75.31, 68.42 (*C*HOH), 55.01 (OMe), 38.91 ($CCMe_2C$), 22.30, 20.60 ($CC(CH_3)_2C$).

4.1.25. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-(pyren-1-ylmethyl)-2,4-dihydroxybutyramide (13a). Compound **12a** (0.95 g, 1.5 mmol) was phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.73 mL, 2.25 mmol) and diisopropylammonium tetrazolide (0.38 g, 2.25 mmol) to afford **13a** (0.945 g, 70%) as a white foam, R_f 0.54, 0.62 ($CHCl_3$–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: m/z=858.3640 $[M+Na]^+$, calcd for $[C_{51}H_{54}N_3O_6P+Na]^+$ 858.3642. ^{31}P NMR (MeCN-d_3) δ 150.71, 149.03 (diastereoisomers, ~1:1). ^1H NMR (MeCN-d_3) δ 8.29–7.90 (m, 9H, Ar*H* (pyrene)), 7.42–7.12 (m, 10H, N*H*, Ar*H* (Dmt)), 6.81 (m, 4H, Ar*H* (Dmt)), 5.10–5.04 (m, 2H, NCH_2), 4.44 (m, 0.5H), 4.37 (m, 0.5H) (C*H*O, diastereomers), 3.70 (c, 6*H*, CH_3), 3.57–2.83 (m, 6H, $POCH_2$, NC*H*, CH_2O), 2.46 (t, 1H, J=6.0 Hz), 2.29 (t, 1H, J=6.0 Hz) (CH_2CN, diastereomers), 2.18 (m, 2H, CH_2CH_2CH), 1.98–0.72 (m, 12H, CH_3 (^{i}Pr)). ^{13}C NMR (MeCN-d_3) δ 172.25, 172.03 (CO), 132.17, 131.79, 131.68, 130.84, 129.49, 128.88, 128.80, 128.68, 128.34, 128.19, 128.11, 128.04, 127.92, 127.57, 127.18, 126.23, 125.70, 125.56, 125.47, 125.33, 124.05, 123.98, 113.83, 86.78, 72.52, 72.40, 71.87, 60.17, 60.07, 59.98, 59.76, 59.54, 59.38, 59.10, 58.94, 55.75, 55.24 (OMe), 46.66, 45.91, 43.89, 43.79, 41.75, 41.61, 34.81, 34.38, 32.24, 24.61, 24.43, 23.28, 23.11, 23.04, 21.91, 20.85, 20.53, 19.62, 14.31.

4.1.26. (2*R*)-O^4-(4,4′-Dimethoxytrityl)-O^2-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)-*N*-(pyren-1-ylmethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (13b). The precursor **12b** (2.65 g, 4.0 mmol) was phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (2.0 mL, 6 mmol) and diisopropylammonium tetrazolide (1.02 g, 6 mmol) to give phosphoramidite **13b** as a white foam (3.16 g, 92%), R_f 0.30, 0.39 (hexane–EtOAc 1:1+1% Et_3N v/v/v). ESI-TOF HRMS: m/z=886.3949 $[M+Na]^+$, calcd for $[C_{53}H_{58}N_3O_6P+Na]^+$ 886.3955. ^{31}P NMR (MeCN-d_3) δ 152.49, 149.36 (diastereomers, ~9:10). ^1H NMR (MeCN-d_3) δ 8.32–7.90 (m, 9H, Ar*H* (pyrene)), 7.45–7.18 (m, 9H, Ar*H* (Dmt)), 6.94 (m, 1H, N*H*), 6.79–6.68 (m, 4H, Ar*H* (Dmt)), 5.02 (m, 2H, NCH_2), 4.04 (d, 0.55H, J_{HCOP}=12.6 Hz), 3.99 (d, 0.45H, J_{HCOP}=9.9 Hz) (C*H*O, diastereomers), 3.70 (m, 6*H*, OCH_3), 3.57–2.75 (m, 6H, $POCH_2$, NC*H*, CH_2O), 2.47 (t, 1.1H, J=6.0 Hz), 2.33 (t, 0.9H, J=6.0 Hz) (CH_2CN, diastereomers), 1.26–0.70 (m, 18H, $CH_2C(CH_3)_2CH$, CH_3 (^{i}Pr)). ^{13}C NMR (DMSO-d_6) δ 169.29 (CO), 129.79, 128.21, 128.16, 127.90, 127.75, 127.70, 127.65, 127.59, 127.40, 127.16, 127.08, 127.04, 126.51, 126.31, 126.24, 125.32, 125.25, 125.21, 125.17, 124.68, 124.57, 124.09, 123.98, 123.95, 123.49, 123.25, 118.76, 118.57, 112.96, 85.13, 85.11, 68.01, 67.66, 58.68, 58.23, 58.19, 55.00 (OMe), 54.96, 44.58, 44.53, 42.69, 42.65, 42.59, 42.55, 24.35, 24.28, 24.23, 24.13, 24.04, 23.98, 22.65, 22.30, 22.22, 21.12, 20.70, 19.84, 19.79, 19.64, 19.42, 19.37.

4.1.27. Solid supports (6a–c) and (14a,b). The solid supports were prepared by a modification of the published method.[10] A solution of succinic anhydride (300 mg, 3 mmol) and DMAP (80 mg, 0.66 mmol) in 10 mL of dry pyridine was added to 300 mg of LCAA-CPG-500 Å, and the mixture was left at room temperature for 24 h with occasional swirling. After filtration, successive washes with 10 mL portions of pyridine, CH_2Cl_2, and Et_2O, and drying, the succinylated LCAA-CPG was suspended in 4 mL of DMF–pyridine (1:1 v/v); compound **4a–c** or **12a**,**b** (0.25 mmol), 1,3-diisopropylcarbodiimide (DIC) (0.28 mL, 1.8 mmol) and DMAP (20 mg) were added; and the slurry was left for 48 h at room temperature. To block the remaining carboxylic acid groups, methanol (0.25 mL) (supports **6a–c**) or a solution of pentachlorophenol (100 mg) in pyridine (1 mL) (supports **14a**,**b**) was added, and the mixture was kept at room temperature for the next 12 h. Then, the support was filtered and for **14a**,**b** only, re-suspended in 5% v/v solution of piperidine in pyridine (3 mL), reacted for 10 min and filtered again. All the supports were washed successively with 10 mL portions of $CHCl_3$, MeOH, MeCN and Et_2O, and dried in vacuo. Loadings were determined by treatment of a portion of the support (5 mg) with 3% w/v CCl_3CO_2H in 1,2-dichloroethane (1 mL) and measuring the absorbance of Dmt^+ at 504 nm (ε=76 mL cm^{-1} μmol^{-1})[10] and were found to be (in μmol/g): 35 (**6a**), 52 (**6b**), 44 (**6c**), 20 (**14a**) and 16 (**14b**).

4.1.28. Synthesis of oligodeoxynucleotides. Oligonucleotide synthesis was carried out on a DNA/RNA synthesiser using standard manufacturer's protocols. Modified phosphoramidites **5a–c**, **10a**,**b** and **13a**,**b** were used in solid-phase oligodeoxyribonucleotide synthesis with their coupling time increased to 10 min. After assembly, the CPG-bound oligonucleotides were treated with concd ammonium hydroxide at 55 °C overnight, evaporated and precipitated from 1 M $LiClO_4$ (0.4 mL) by dilution with acetone (1.6 mL). Oligonucleotides were isolated by electrophoresis in 20% denaturing (7 M urea) polyacrylamide gel in Tris–borate buffer, pH 8.3, and desalted using NAP-10 column and standard purification procedure.

RP-HPLC of oligonucleotides was carried out using a Phenomenex RP-C18 column (3.90×300 mm) and dual-wavelength (215 and 254 nm) UV detection using gradient of acetonitrile in 0.1 M aq triethylammonium acetate (0–5%, 5 min, 5–15%, 10 min, 15–40%, 30 min, 40–80%, 10 min, 80–0%, 10 min).

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