Synthesis of new phosphonoglycine backbone units for the development of phosphono nucleic acids.

Bogdan Doboszewski,^[a,b] Elisabetta Groaz,^[b] and Piet Herdewijn*^[b]

Keywords: Nucleotides / Phosphonates / Phosphono peptide nucleic acid (pPNA)

A series of phosphono-modified backbone mimics based on achiral and chiral *N*-(dihydroxypropyl)glycine units were obtained by sequential addition of phosphonate and nucleobase moieties to suitably protected dihydroxypropylamines. Simple synthetic strategies enabled the preparation of various target derivatives that will be useful as building blocks for the preparation of new synthetic polymers containing a phosphonate internucleotide linkage in place of the standard phosphodiester bond.

Introduction

Genetic information in all living organisms is coded in sequences of pyrimidines and purines connected to a backbone of phosphorylated 2-deoxy-(*D*)-ribofuranoses (in DNA) or (*D*)-ribofuranoses (in RNA). The development of unnatural nucleic acids, especially comprising sugar mimics in their backbone replacing the natural furanosyl units, for the formation of new oligomeric pairing systems represents an important avenue of research in synthetic biology and in the search for the origin of life.^[1] Studies aiming at defining an etiology of nucleic acids have demonstrated that canonical Watson-Crick base pairing can be supported by chemically simplified backbone structures.^[2-4] Among such modifications, one relevant class of DNA mimics consists of Peptide Nucleic Acids (PNAs),^[5] linear pseudo-peptide backbones connected to nucleobases through methylene carbonyl linkages. Many structural variants, closely related to *N*-(2-aminoethyl)-glycine PNA,^[6] were synthesized and found to be capable of stable antiparallel homoduplex and triple helix formation (Figure 1).^[5] PNA strongly hybridizes with natural complements in a sequence-specific manner, forming PNA·DNA and PNA·RNA binary complexes with increased specificity and thermal stability compared to unmodified homoduplexes, owing to its nonionic nature.^[7] When it comes to in vivo application, a significant drawback regarding PNAs as biomolecular tools arises from their inadequate membrane permeability.

An effective solution to the above critical problem requires the development of structurally distinct oligomers. Up to now, few independent literature reports described autonomous pairing systems built upon *N*-(2-hydroxyethyl)phosphonoglycine (*O*-pPNAs) or *N*-(2-aminoethyl)phosphonoglycine (*N*-pPNAs) backbones (Figure 1).^[8-12] Although such family of mimics benefits from the insertion of a phosphono-ester unit in their backbones, which gives them a better metabolic stability, they display

considerably weaker hybridization affinity for cognate DNA and RNA as compared to PNAs and natural oligonucleotide counterparts. A certain improved ability for heteroduplex formation resulted from chimeric sequences alternating pPNA and PNA/PNA-like monomers.^[13-15]

Figure 1. Repeating monomeric units of DNA, PNA and its closely related DNA mimics (selected examples).

Given our long-standing interest in the search for novel artificial oligonucleotides, we set out to design and synthesize modified phosphono building blocks **1a-c** and **2** (Figure 2) as precursors to a new generation of polymers. We are investigating the possibility that a supplementary hydroxyl group in the backbone of pPNAs might facilitate groove solvation and thus prove to be advantageous upon binding with DNA and/or RNA targets. In the proposed structures, the internucleotide linkers contain 7 or 6 bonds dependent on which hydroxyl group is used for ester formation, and both are compatible with hybridization properties.^[16] Here, we present our synthetic efforts to the preparation of achiral and chiral phosphonoglycol monomers depicted in Figure 2.

Figure 2. General chemical structures of planned monomeric targets.

[a] Departamento de Química, Universidade Federal Rural de Pernambuco, 52171-900 Recife, PE, Brasil

[b] Laboratory for Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

E-mail: Piet.Herdewijn@rega.kuleuven.be

Homepage: <http://medchem.rega.kuleuven.ac.be/>

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/ejoc.xxxxxxxx>.

Results and Discussion

From a strategic point of view, it was first decided that the target building blocks **1a-c** and **2** should be assembled by stepwise addition of phosphonate and nucleobase moieties to appropriately protected amino dihydroxy synthons. Preliminary efforts were directed towards the preparation of the key racemic glycol derivative **6**, which would serve as precursor for **1a** (Scheme 1). Thus, selective *N*-benzoylation^[17, 18] of 3-amino-1,2-dihydroxy propane **3**, followed by tritylation of compound **4**, generated secondary alcohol **5**, which, upon harsh basic hydrolysis, afforded 3-amino-1-*O*-trityl-2-propanol **6** in good overall yield.

Scheme 1. Reagents and conditions: i) BzCl, K₂CO₃, H₂O. ii) TrCl, Py. iii) 4N aq. NaOH, EtOH, Δ.

Next, in order to obtain both enantiomeric forms of the above aminoalcohol, different routes were explored. The known (*S*)-1-*O*-trityl-2,3-propanediol **7**, previously obtained from 5-*O*-trityl-(*D*)-arabinofuranose,^[19] was our initially chosen substrate. However, conventional tosylation of **7** proceeded with surprisingly little selectivity, and a mixture of the desired 3-*O*-tosylate **8** (54%) and 2,3-bis-*O*-tosylate **9** (23%) was formed (Scheme 2). More importantly, **8** and **9** were difficult to separate by chromatography due to very similar R_fs. In view of those difficulties, we resorted to the tin methodology.^[20] Treatment of diol **7** with Bu₂SnO followed by TsCl, permitted isolation of the required monotosylate **8** in 97% yield over two steps.

Scheme 2. Reagents and conditions: i) TsCl, Py. ii) Bu₂SnO, MeOH. iii) TsCl, THF. iv) NaN₃, DMF.

Subsequent substitution using NaN₃ furnished chiral azide (*S*)-**11** in 70% yield, while the methodology described by Kutney *et al.*^[21] via transient cyclic carbonate **12** or Mitsunobu reaction^[22] using **7** and diisopropylazodicarboxylate/Ph₃P/HN₃ were both unsuccessful. Both compounds **6** and **11** were abandoned later due to difficulties during a reduction of the azide function, during a phosphorylation step and subsequent coupling with thymine-1-yl acetic acid (see below).

As an alternative route, we decided to turn to commercially available (*R*)-glycidol **13** as precursor to our desired chiral synthon.

After silylation of **13** at the primary position,^[23] the resulting protected epoxide derivative **14** (which can also be obtained by kinetic resolution^[24] or by asymmetric epoxidation^[25]) underwent noticeable decomposition during its ring opening to azido alcohol **15** (Scheme 3) and only marginal quantity of the expected compound **15** (ca 3%) could be isolated. Pleasingly, the replacement of **13** with (*S*)-1,2-isopropylidene-glycerol (*S*-Solketal) **16** as starting material led to much improved results in terms of isolated yields. Compound **16** and its enantiomer are produced in ton scale by desymmetrization of 1,2-isopropylidene-glycerol hydrogen phthalate using chiral 1-phenylethylamine.^[26]

Scheme 3. Reagents and conditions: i) TBDPSCl, Et₃N, CH₂Cl₂. ii) NaN₃, DMF, 78 °C. iii) TsCl, Py. iv) NaN₃, DMF. v) *p*TSA-H₂O, MeOH. vi) TBDPSCl, Im, DMF. vii) Ac₂O, Py. viii) Ph₃P, H₂O. ix) Ph₃P, THO, H₂O or Na₂S·9H₂O.

Following tosylation of the hydroxyl group on **16**^[27] and substitution using NaN₃,^[28] hydrolysis of the isopropylidene protection upon treatment with *p*TSA-H₂O^[28, 29] and selective silylation at the primary hydroxyl group generated the desired (*S*)-3-azido-1-*t*-butyldiphenylsilyloxy-2-propanol **15** in good yield (82%). This material was identical to that prepared through the previous approach. Acetylation of **15** proceeded quantitatively, but attempted conversion of the azide into the required amino function to give **21** via Staudinger reaction^[30] (Ph₃P, wet THF) resulted in a mixture of products presumably resulting from migration of the acetyl group and possibly of the silyl group as well. Consequently, compound **20** as a precursor of **1b** was abandoned. Evidently, a non-migrating hydroxyl-protecting group in **15**, as well as in **11**, like a benzyl group, must be used. This remains to be investigated. In light of these results, we decided to use (*S*)-azide **18** directly as such instead of transforming it into hydrophobic compound **20**. Treatment of **18** with either Na₂S·9H₂O^[31] or more conveniently under Staudinger reaction conditions^[30] accomplished the reduction of the azido function, affording the corresponding amine (*S*)-**22**^[32, 33] in 70% isolated yield in both cases. The amine **22** is very polar and inconvenient to handle on a small scale. Its advantage however consists in the liberation of both hydroxyl groups in a single deprotection step (see below).

Although the corresponding (*R*)-3-amino-1,2-*O*-isopropylideneoxy propane, could be obtained by repeating the same synthetic sequence shown in Scheme 3, starting from readily available enantiomeric (*R*)-Solketal,^[26] we devised a different approach based on (*D*)-galactose (Scheme 4). (*D*)-Galactose is the only easily available sugar that can be converted in one step into its derivative **23**, which exposes a primary hydroxyl group. We reasoned that this compound could be transformed into a 6-amino-6-deoxy-(*D*)-galactopyranose derivative **28**, which in turn could be degraded by NaIO₄ to form (*R*) amine **29** in which the stereogenic atom C2 would be the C5 atom in its predecessor **27**. Galactose does not seem to be much exploited in this sense.^[34]

Scheme 4. Reagents and conditions: i) TsCl, Py. ii) NaN₃, DMF, 120 °C. iii) Ph₃P, THF, H₂O.

The logic of this procedure in terms of “economy of. chirality” can be questioned since only one stereogenic center present in the substrate **27** is used, and three others are lost. Nevertheless, ease of accessibility to 1,2;3,4-di-*O*-isopropylidene-*(D)*-galactopyranose **23**^[35] prompted us to use it as described above. Commercially available **23** was converted into its 6-*O*-tosylate **24**^[35] which after treatment with an excess of NaN₃ furnished azide **25**.^[36] This apparently simple substitution at the primary position of **24** is known to require extended reaction time, elevated temperatures and excess of nucleophile. The use of a triflate derived from **23** rather than tosylate **24** makes substitutions much easier.^[37–39] The azide **25** was reduced to the amine **26** in nearly quantitative yield using again Staudinger conditions. The same compound was previously obtained using catalytic hydrogenation^[36] of **25** or lithium aluminum hydride reduction of the corresponding 6-oxime.^[40]

The last amine necessary for the synthesis of achiral building block **2** was easily obtained by standard silylation of 2-amino-1,3-propanediol (Scheme 5). An insignificant amount of monosilylated compound **32** was also formed together with the desired 2-amino-1,3-bis-*t*-butyldiphenylsilyloxy propane **31**.

Scheme 5. Reagents and conditions: i) TBDPSCl, Im, DMF.

Having successfully obtained the desired protected amines **6**, **22**, **26** and **31** in workable quantities, the subsequent transformation for installing the phosphonate group was achieved using standard alkylation conditions, as illustrated in Table 1.

The required alkylating reagents **33a**^[41] (R'=SO₂C₆H₄CH₃) and **33b**^[42] (R'=SO₂CF₃) were prepared according to literature procedures. The reaction of diethylphosphonomethyl tosylate **33a** with primary amines **22** and **26** gave phosphonomethylamines **34** and **35** in moderate yields (~20 to 30%), while the amine **31** underwent alkylation with more reactive triflate **33b** in good yield (~56%). Phosphonylation of racemic amine **6** with **33b** was possible, but this reaction was complicated by facile double alkylation at NH₂, which led to mono-*N*-alkylated derivative **37** (18%) together with 22% of doubly phosphonomethylated product **38**. Consequently, it proved necessary to turn to another substrate, such as commercially available 3-amino-1,2-isopropylidenoxy propane **39**, which gave 30% yield of alkylated product. It should be mentioned that attempts to increase yields by using diethylphosphonomethylsulfonimidazole in the place of **33a** or **33b** were uniformly unsuccessful. The sulfonimidazole moiety

was claimed to be a better leaving group than a tosylate/mesylate but inferior to triflate,^[43] and can be obtained *in situ* by reacting (EtO)₂P(O)CH₂OH with sulfonyl diimidazole^[44] and NaH, or with sulfonyl chloride and imidazole.^[43] Unexpectedly, subsequent addition of amine produced no traces of the desired products.

Table 1. Synthesis of phosphonomethylamines **34–38**, **40**.

entry	amine	R'	product	yield
1		Ts		21.5%
2		Ts		37%
3		Tf		56.6%
4		Tf		18%
				22%
5		Ts		30.2%

In the following step, phosphonomethylamines **34**, **35**, **36** and **40** were reacted in the presence of DCC and hydroxybenzotriazole with thymine-1-yl acetic acid **41** (Scheme 6), obtained in turn by alkylation of thymine with chloroacetic acid in basic medium.^[45] The corresponding amides were generally produced in excellent yields, the only exception to this trend being the amide derived from amine **36**, most probably due to the steric hindrance of the bulky silyl groups.

Scheme 6. Reagents and conditions: i) DCC, HOBt, DMF.

At this point, compound **43** was subjected to further manipulation *via* the deprotection-degradation sequence discussed earlier (Scheme 7). First, the acetonide protecting groups were removed by exposure to aqueous TFA, leading to tetraol **46** in 86% yield. Next, this compound was reacted with four equivalents of sodium periodate under carefully controlled conditions. We recognized that the resulting transient aldehyde would be prone to fast decomposition, therefore it was immediately reduced upon treatment with sodium borohydride. Gratifyingly, the desired diol **47** could be isolated in reasonable yield (52%), together with minor amounts of two degradation fragments, presumably formed in the initial oxidation step. The reaction that concluded our synthesis

was a dealkylation using trimethylsilyl iodide (TMSI) and furnished the (*R*)-monomer **50**, a precursor of **1c**.

Scheme 7. Reagents and conditions: i) 80% aq. TFA. ii) NaIO₄, EtOH, H₂O. iii) NaBH₄, EtOH. iv) TMSI, DMF; ion exchange chromatography (1M TEAB:H₂O).

TMSI acting in turn on compounds **42** and **45**, allowed removal of the isopropylidene acetal and cleavage of the diethoxy ether groups in a single synthetic operation, as depicted in Scheme 8. Purification on silica gel, followed by anion exchange chromatography completed access to phosphonates **51** and **52**, precursors of **1b** and **1a** respectively.

Scheme 8. Reagents and conditions: i) TMSI, Et₃N, DMF; ion exchange chromatography (1M TEAB:H₂O).

Bis-silylated phosphonamido thymine **44** was initially treated with TBAF to effect liberation of the hydroxyl functionalities. Unfortunately, this approach invariably provided a complex mixture of inseparable products, most likely due to the sensitivity of the substrate to a basic media. The same outcome was obtained using HF/pyridine complex. After those unsuccessful attempts, we found that the use of a milder reagent such as tris(dimethylamino)sulfonium difluorotrimethylsilicate (TAS-F)^[46] was critical to avoid formation of undesired by-products, mainly arising from preferential formation of cyclic phosphonates. Finally, the phosphono functionality of **53** was deprotected by the action of TMSI (Scheme 8) to give monomer **54**.

Scheme 9. Reagents and conditions: i) TAS-F, DMF, 0 °C to rt. ii) TMSI, Et₃N, DMF; ion exchange chromatography (1M TEAB:H₂O).

Conclusions

In summary, synthetic routes have been developed for the preparation of new backbone motifs, comprising of N-alkylated phosphonoglycine moieties. These monomers will be used for constructing new unnatural nucleic acids with a phosphonate internucleotide linkage.

Experimental Section

General methods. NMR spectra were recorded on Bruker Avance 300 (1H 300 MHz, 13C 75 MHz, 31P 121 MHz) or Avance II 500 (1H 500 MHz, 13C 125 MHz, 31P 202 MHz) spectrometers. Spectra were referenced to TMS and chemical-shift (δ) values, expressed in parts per million (ppm), are reported downfield of TMS. The multiplicity of signals is reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or a combination of any of these. Coupling constants are expressed in hertz (Hz). For final products, 1H and 13C resonance assignments were made with the aid of 1H–1H and 1H–13C correlation experiments. High resolution mass spectra (HRMS) were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA), utilizing electrospray ionization (ESI). Samples were infused at 3 μ L/min and spectra were obtained in positive (or negative) ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass. Chromatographic separations were performed with silica gel (100–200 mesh or 230–400 mesh) and analytical thin-layer chromatography was performed on precoated aluminum backed sheets (254 nm) supplied by Fluka. For all reactions, analytical grade solvents were used. Anhydrous THF was refluxed over sodium-benzophenone ketyl and distilled. Dry dichloromethane was obtained by distillation over CaH₂ and dry pyridine was refluxed and stored over KOH. Dry dimethylformamide was supplied by Aldrich. All final compounds were purified by anion exchange chromatography (Source 15Q), using H₂O and 1M TEAB as eluents. Optical rotations were recorded on a Perkin–Elmer 341 polarimeter in a thermostated cell of length 1 dm at 20 °C using the sodium D-line, and a suitable solvent that is reported along with the concentration (in g/100 ml).

(*Rac*)-3-*N*-Benzamido-1-triphenylmethoxy-2-propanol (5**):** To a cold (ice-water bath) solution of 3-amino-1,2-propane diol (9.1 g, 100 mmol) in H₂O (70 ml) was added K₂CO₃ (30 g) followed by benzoyl chloride (13.5 ml, 16.3 g, 116 mmol) dropwise. The mixture was then magnetically stirred overnight at 40 °C. The product **4** has R_f 0.36 in CH₂Cl₂-MeOH 10:0.7 (staining with ninhydrin). The reaction mixture was quenched by addition of 1M HCl and subsequently evaporated to dryness. The resulting residue was resuspended in ethanol, the mixture was then filtered and the filtrate was evaporated under reduced pressure. Chromatography in CH₂Cl₂-MeOH (gradient) 10:0.7 $\rightarrow$ 10:1 furnished **4** (19.4 g, quantitatively) as a colorless syrup.^[17, 18] The NMR data match those published before.^[18] This compound (20.0 g, 102 mmol) in dry CH₂Cl₂ (100 ml) and pyridine (35 ml) was cooled in an ice-water bath under argon atmosphere, and TrCl (32.0 g, 115 mmol) was added in one portion while maintaining magnetic stirring. Cooling was removed and the reaction mixture was left overnight at r.t., followed by 48 h at gentle boiling. TLC showed the product **5** R_f 0.55 in hexane-EtOAc 1:1, yellow using CrO₃-H₂SO₄ system, or pink using ninhydrin. Extraction (CH₂Cl₂-H₂O), drying, evaporation and coevaporation with xylenes, resulted in spontaneous crystallization. The crystals were filtered, washed with hexane and dried to yield **5** (31.5 g, 70%), m.p. 170–174 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ = 8.33 (t, *J* = 5.5 Hz, exchangeable, 1 H), 7.29 (d, *J* = 6.9 Hz, 2 H), 7.77–7.21 (m, aromatic, 18 H), 5.12 (d, *J* = 5.3 Hz, exchangeable, 1 H), 3.92 (m of 6 lines, *J* = 5.6 Hz, 1 H), 3.53–3.45 and 3.29–3.20 (two m, 1 H each), 3.01 (dd, *J* = 5.5, 9.5 Hz, 1 H), 2.97 (dd, *J* = 5.0, 9.0 Hz, 1 H) ppm. After D₂O exchange: δ = 3.91 (m of 5 lines, *J* = 5.7 Hz), 3.37 (dd, *J* = 5.3, 13.4 Hz), 3.25 (dd, *J* = 7.1, 13.3 Hz) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆): δ = 166.4, 143.9, 134.5, 131.0, 128.3, 128.1, 127.8, 127.2, 126.9, 85.8, 68.5, 66.2, 45.4 ppm. HRMS (pos.): calcd. for C₂₉H₂₇NNaO₃ [M+Na]⁺ 460.1883; found 460.1876.

(Rac)-3-Amino-1-triphenylmethoxy-2-propanol (6):

Compound **5** (2.0 g, 4.5 mmol) in EtOH (40 ml) and 4N NaOH (30 ml) was stirred under reflux during 48 h. Water (60 ml) was added and the resulting heterogeneous mixture was kept in ice-water for 1 h. Precipitated colorless material was filtered, washed with ice-water, dried on oil pump and solubilized in EtOH (50 ml) with warming. Some solid material was removed by filtration and volatiles were evaporated. TLC showed a spot of the product R_f 0.40 (CH₂Cl₂-MeOH-25% NH₄OH 20:2:0.1), purple with ninhydrin or yellow with Hanessian's stain. Chromatography in the same system furnished **6** (1.1 g, 72%) as a glassy material. Addition of Et₂O resulted in the formation of a colorless powder that has a melting range *ca* 77°–*ca* 115 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ = 7.45–7.22 (m, aromatic, 15 H), 3.67–3.59 (m unresolved, 1 H), 3.01 (dd, J = 5.5, 9.0 Hz, 1 H), 2.90 (dd, J = 6.0, 9.0 Hz, 1 H), 2.71 (dd, J = 4.1, 12.7 Hz, 1 H), 2.51 (dd, J = 7.1, 12.6 Hz, 1 H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆): δ = 143.9, 128.3, 127.8, 126.9, 85.7, 71.1, 65.9, 45.3 ppm. HRMS (pos.): calcd. for C₂₂H₂₄NO₃ [M+H]⁺ 334.1801; found 334.1819.

(R)-1-Tosyloxy-3-triphenylmethoxy-2-propanol (8) and (R)-1,2-bis-tosyloxy-3-triphenylmethoxy propane (9): (S)-Diol **7**^[19] (0.25 g, 0.75 mmol) in dry CH₂Cl₂ (15 ml) and Et₃N (0.3 ml) was treated with TsCl (0.19 g, 1 mmol) during 18 h under protective atmosphere (argon). TLC showed two closely positioned compounds (R_f of a mid-point 0.38 in hexane-EtOAc 140:55). Extraction (CH₂Cl₂-H₂O), drying (MgSO₄), evaporation and flash chromatography yielded **9** (0.11 g, 23%) and **8** (0.197 g, 54%). Data for **9**: foam, α_D = -2.1° c 3.2 dioxane. ¹H NMR (300 MHz, CDCl₃): δ = 7.70–7.60 (m, aromatic, 4 H), 7.25 (m, aromatic, 15 H), 4.55 (m of 5 lines, J = 4.9 Hz, 1 H), 4.15–4.13 (m unresolved, 2 H), 3.28 (dd, J = 5.2, 10.0 Hz, 1 H), 3.23 (dd, J = 4.9, 10.0 Hz, 1 H), 2.43 (s, 6 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 145.3, 145.2, 143.3, 133.3, 132.4, 130.1, 130.0, 128.7, 128.2, 128.2, 128.1, 127.4, 87.4, 77.5, 67.8, 62.1, 21.9 ppm. HRMS (pos.): calcd. for C₃₆H₃₄NaO₇S₂ [M+Na]⁺ 665.1638; found 665.1634. Data for **8**: foam, α_D = -11.3° c 1.8 dioxane. ¹H NMR (300 MHz, CDCl₃): δ = 7.76 (d, J = 8.2 Hz, 2 H), 7.37–7.22 (m, 17 H), 4.15 (dd, J = 4.4, 10.2 Hz, 1 H), 4.06 (dd, J = 6.0, 10.1 Hz, 1 H), 3.92 (m of 6 lines, J = 5.2 Hz, 1 H), 3.21 (dd, J = 5.3, 9.7 Hz, 1 H), 3.16 (dd, J = 5.5, 9.8 Hz, 1 H), 2.42 (s, 3 H), 2.35 (d, J = 5.6 Hz, 1 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 145.2, 143.6, 132.8, 130.1, 128.7, 128.2, 128.1, 127.4, 87.1, 71.2, 68.9, 63.8, 21.8 ppm. HRMS (pos.): calcd. for C₂₉H₂₈NaO₅S [M+Na]⁺ 511.1549; found 511.1554.

Compound **8** *via* tin oxide methodology^[20]: Diol **7** (0.24 g, 0.71 mmol) in dry toluene (20 ml) and dibutyltin oxide (0.23 g, 0.92 mmol) were magnetically stirred at gentle reflux under argon. The mixture became homogenous after *ca* 20 min. After a total of 80 min the mixture was cooled down, evaporated and dried on oil pump. The vacuum was broken using dry argon in a balloon. To product **10** thus obtained was added dry THF (10 ml) followed by TsCl (0.15 g, 0.8 mmol), and this mixture was left overnight at rt. TLC showed the monotosylate **8** as the only product. Evaporation of the solvent and flash chromatography in hexane-EtOAc 7:3 furnished **8** (0.25 g, 97%).

(S)-1-Azido-3-triphenylmethoxy-2-propanol (11): Tosylate **8** (0.13 g, 0.26 mmol) in dry DMF (10 ml) and NaN₃ (0.65 g, 10 mmol) were stirred under argon at 88 °C for 2.5 h. TLC showed new intensely charring spot (Hanessian's stain) less polar than a substrate R_f 0.64, hexane-EtOAc 7:3 (substrate's R_f is 0.40). Extraction (CH₂Cl₂-H₂O), drying (MgSO₄), filtration, evaporation and purification by preparative TLC plate using hexane-EtOAc 4:1, furnished glassy product (0.067 g, 70%). α_D = -8.4° c 3.4 CHCl₃. ¹H NMR (300 MHz, CDCl₃): δ = 7.42–7.23 (m, aromatic, 15 H), 3.88

(unresolved t, J = 5.3 Hz, 1 H), 3.35 (d, J = 5.3 Hz, 2 H), 3.20 (d, J = 5.3 Hz, 2 H), 2.39 (br s, 1 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 143.5, 128.5, 127.9, 127.2, 86.9, 70.1, 64.7, 53.8 ppm. HRMS (pos.): calcd. for C₂₂H₂₁N₃NaO₂ [M+Na]⁺ 382.1526; found 382.1532. Attempts to obtain the azide **11** *via* a cyclic carbonate **12** by analogy to the other substrates^[21] (diol **7** and carbonyldiimidazole, followed by NaN₃) or using a Mitsunobu reaction^[22] (**7**, PPh₃, HN₃, *i*PrO₂CNNCO₂*i*Pr) were negative.

(S)-3-Azido-1-*t*-butyldiphenylsilyloxy-2-propanol (15): Method a. Starting from (R)-glycidol: (R)-glycidol (Sigma-Aldrich) **13** was conventionally silylated^[23] to furnish **14** as an oil in *ca* 90% yield after chromatography (gradient, hexane-Et₂O 10:0.5→10:1.5), R_f 0.47 in hexane-Et₂O 10:1, α_D -1.9° c 21.6, CHCl₃, lit. α_D -1.4° c 1 CHCl₃,^[24] α_D -2.3° c 9.07 CHCl₃.^[25] (Optical rotation is not presented in the ref. 13). This compound (1.21 g, 3.9 mmol) in dry DMF (24 ml) and NaN₃ (2.0 g, 30 mmol) was stirred under argon at 78 °C during 8 h. The mixture turned brown. TLC showed the product **15** R_f 0.34 in hexane-Et₂O 4:1, along with the decomposition products. Extraction (CH₂Cl₂-water), drying, evaporation of solvent and chromatography (gradient, hexane-Et₂O, 20:4→20:6) furnished **15** (0.049 g, *ca* 3%) as an oil. ¹H NMR (300 MHz, CDCl₃): δ = 7.66–7.35 (m, two groups of signals, aromatic, 10 H), 3.86 (m of 6 lines, J = 5.2 Hz, 1 H), 3.71–3.62 (m, 2 H), 3.56 (apparent d, J = 5.5 Hz, 2 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 135.7, 133.0, 130.2, 128.1, 71.1, 65.2, 53.4, 27.0, 19.4 ppm. HRMS (pos.): calcd. for C₁₉H₂₅KN₃O₂Si [M+K]⁺ 394.1353; found 394.1347. Method b. Starting from (S)-1,2-isopropylidene glycerol *via* (S)-3-azido-1,2-propane diol **19**: The diol **19** (2.55 g, 21.8 mmol) in dry DMF (30 ml) and imidazole (3.2 g, 48 mmol) was treated with *t*BuPh₂SiCl (6.6 g, 6.2 ml, 14 mmol), added dropwise using a syringe during *ca* 5 min with cooling in ice bath and under argon atmosphere. After addition was complete, the reaction proceeded at r.t. during 6 h. TLC showed a spot R_f 0.33 (hexane-Et₂O 4:1). Extraction (CH₂Cl₂-H₂O), drying, evaporation and chromatography in hexane-Et₂O 3:1 furnished **15** (6.4 g, 82%) as an oil.

(S)-3-Azido-1,2-propanediol (19): Commercial (S)-2,3-isopropylidene glycerol **16** (Fluka) (15.4 g, 116.3 mmol) was dissolved in CH₂Cl₂ (150 ml), Et₃N (25 ml) and TsCl (16.5 g, 139 mmol) were then added. The mixture was stirred for 8 h, isolation after conventional extraction and chromatography in hexane-EtOAc 4:1, yielded **17**, R_f 0.45, (31.6 g, 95%), α_D -3.6° c 6, EtOH, lit.^[27] -4.5° c 1, EtOH. Compound **17** (10.0 g, 35 mmol) was then reacted with NaN₃ (11.3 g, 175 mmol) in dry DMF (120 ml) at 65° C for 20 h. After extraction and chromatography in hexane-Et₂O 4:1, R_f 0.43, azide **18** was isolated (5.3 g, 96.5%), α_D -52.2° c 5.6 CHCl₃, lit.^[28] α_D not given]. This azide (5.1 g, 32.5 mmol) was dissolved in MeOH (360 ml) and *p*TSA monohydrate (0.32 g, 1.7 mmol) was added, the mixture was kept at r.t. during 25 h.^[28] TLC showed more polar product R_f 0.40 hexane-EtOAc 1:3 which chars with a Hanessian's stain. Neutralization with solid NaHCO₃, 5.3 g during 45 min., filtration, evaporation and chromatography in hexane-EtOAc 1:3 furnished **19** as an oil (2.8 g, 57%), α_D +2.8° c 4.7 CHCl₃, α_D -16.2° c 3.6, MeOH; lit.^[29] α_D -10.8° c 0.98 MeOH. ¹H NMR (300 MHz, DMSO-*d*₆): δ = 5.09 (d, J = 5.1 Hz, exchangeable, 1H), 4.67 (t, J = 5.6 Hz, exchangeable, 1 H), 3.68–3.59 (m of 15 lines, 1 H), 3.41–3.14 (m of 16 lines, 4 H) ppm. After D₂O exchange: 3.66–3.59 (m of 12 lines, 1 H), 3.36 (dd, J = 5.4, 10.9 Hz, 1 H), 3.28 (dd, J = 6.6, 10.6 Hz, 1 H), 3.26 (dd, J = 3.9, 12.7 Hz, 1 H), 3.16 (dd, J = 6.7, 12.7 Hz, 1 H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆): δ = 74.0, 63.2, 53.5 ppm.

(S)-2-Acetoxy-3-azido-1-*t*-butyldiphenylsilyloxy propane (20): Conventional acetylation of **15** (4.71 g, 13.3 mmol) in pyridine (30

ml) and Ac₂O (15 ml) overnight, followed by extractive work-up, coevaporation with xylenes and exhaustive drying on oil pump furnished **20** as a syrup (5.3 g, quantitatively), R_f 0.63 in hexane-Et₂O 4:1, α_D -14.6°, c 2.8 CHCl₃. ¹H NMR (300 MHz, CDCl₃): δ = 7.68-7.35 (m, two groups of signals, 10 H), 5.06 (m of five lines, J = 5.2 Hz, 1 H), 3.79 (dd, J = 5.0, 10.9 Hz, 1 H), 3.74 (dd, J = 5.5, 10.9 Hz, 1 H), 3.53 (apparent d, J = 5.2 Hz, 2 H), 2.04 (s, 3 H), 1.06 (s, 9 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 170.3, 135.8, 135.7, 133.1, 130.1, 128.0, 127.9, 72.8, 62.6, 50.9, 26.9, 21.1, 19.4 ppm. HRMS (pos.): calcd. for C₂₁H₂₇N₃NaO₃Si [M+Na]⁺ 420.1714; found 420.1740. Attempted conversion of the azide **20** to get amine **21** via a Staudinger reaction^[30] resulted in a complex reaction mixture, which was not investigated.

(S)-3-Amino-1,2-isopropylidenoxy propane (22): This compound was obtained by reduction of the azide **18** using either a Staudinger reaction^[30] or Na₂S₉H₂O by analogy to the published procedure.^[31] Via a Staudinger reaction: To a magnetically stirred solution of **18** (1.6 g, 10 mmol) in THF (20 ml) and water (4 drops) was added Ph₃P (3.1 g, 12 mmol). Evolution of nitrogen started within ca 30 s. After an overnight reaction the product showed a spot visualized with ninhydrin R_f 0.50 in CH₂Cl₂-MeOH-25%NH₄OH 20:2:1. Evaporation of volatiles and chromatography in the abovementioned system furnished **22** as yellowish oil (1.06 g 70%). Via Na₂S₉H₂O: To a solution of azide **18** (3.35 g, 21.3 mmol) in MeOH (10 ml) was added a solution of Na₂S₉H₂O (6.4 g) in H₂O (10 ml). Stirring was continued for 16 h at 55 °C. The reaction mixture was transferred to a separatory funnel charged with Et₂O and H₂O. After exhaustive extraction with Et₂O (six times), the organic phase was dried and the solvent was evaporated to furnish an yellowish oil. Crude **22** thus obtained was used for the subsequent transformation. α_D +1.1° c 14, CHCl₃, lit.^[33] α_D +0.9° c 1 CHCl₃, lit.^[32] α_D +1.48° neat. ¹H NMR (300 MHz, CDCl₃): δ = 4.13 (ddd, J = 4.3, 6.3, 12.6 Hz, 1 H), 4.04 (dd, J = 6.4, 7.9 Hz, 1 H), 3.52 (dd, J = 6.4, 7.9 Hz, 1 H), 2.85 (dd, J = 4.4, 13.1 Hz, 1 H), 2.78 (dd, J = 7.1, 13.1, 1 H), 1.43 (s, 3 H), 1.36 (s, 3 H), 1.26 (br s, 2 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 109.2, 77.5, 67.0, 44.8, 26.9, 25.4 ppm.

6-Amino-6-deoxy-1,2;3,4-di-O-isopropylidene-(D)-galactopyranose (26):

Conventional tosylation of commercial 1,2;3,4-di-O-isopropylidene-(D)-galactopyranose **23** (α_D -56.8°, c 5.9 CHCl₃, Acros) in CH₂Cl₂, pyridine or Et₃N and TsCl (1.1 eq) overnight, followed by extractive work-up (CH₂Cl₂-H₂O) and chromatography in hexane-EtOAc 3:1, R_f 0.41, furnished **24**^[35] (89%), α_D -62.4°, c 1.7 CHCl₃, lit.^[35] α_D -63°, CHCl₃, c not given. No attempt was made to crystallize this compound.^[35] Tosylate **24** was converted to the corresponding 6-azide **25** by analogy to the procedure for (L)-galactose^[36] (NaN₃ 5 eq, DMF, ca 120° C, 18 h under argon) to furnish **25** in 91% yield after extractive work-up (CH₂Cl₂-H₂O) and chromatography in hexane-EtOAc 5:1, R_f 0.39; α_D -98°, c 2.4 CHCl₃. To a magnetically stirred solution of this azide (3.3 g, 11.6 mmol) in THF (50 ml) was added H₂O (0.3 ml) followed by Ph₃P (3.7 g, 14 mmol) by analogy to similar reduction.^[30] Evolution of nitrogen started in ca 30 s. Next day TLC showed that all substrate reacted to form the amine **26** R_f 0.61 in CH₂Cl₂-MeOH 20:3. The solvent was evaporated and the residue was purified by chromatography (gradient) CH₂Cl₂-MeOH 20:2→20:3 to furnish **26** as an oil (2.94 g, 98%). Alternatively, **25** was subjected to catalytic hydrogenation (10% Pd/C, EtOH, at 50 psi in a Parr apparatus during 6 h) to furnish amine **26** in nearly quantitative yield. α_D -42.1°, c 1.4, MeOH; lit.^[40] -53.1° c 1 CHCl₃; α_D +48.6° c 1.4 MeOH for the (L)-form.^[36] ¹H NMR (300 MHz, CDCl₃): δ = 5.56 (d, J = 5.0 Hz, 1 H), 4.60 (dd, J = 2.3, 7.9 Hz, 1 H), 4.32 (dd, J = 2.4, 5.0 Hz, 1 H), 4.23 (dd, J = 1.9, 7.9 Hz, 1 H), 3.84 (ddd, J = 1.7, 4.6, 7.2 Hz, 1 H), 3.54 (br s, 2 H, in the other

samples this signal appeared at 2.43 ppm and 2.22 ppm), 3.04 (dd, J = 8.0, 13.2 Hz, 1 H), 2.96 (dd, J = 4.1, 13.2 Hz, 1 H), 1.57 (s, 3 H), 1.45 (s, 3 H), 1.33 (s, 6 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 109.5, 108.9, 96.5, 71.8, 71.0, 70.7, 68.3, 41.9, 26.3, 26.2, 25.1, 24.5 ppm.

2-Amino-1,3-bis-(*t*-butyldiphenylsilyloxy) propane (31) and (rac)-2-amino-1-*t*-butyldiphenylsilyloxy-3-propanol (32): 2-Amino-1,3-propane diol (Acros or Aldrich) (3.85 g, 42.8 mmol) was solubilized in dry DMF (36 ml) under argon. To this solution was added imidazole (12.6 g, 185.1 mmol) followed by *t*BuPh₂SiCl (24.7 g, 23.3 ml, 90.1 mmol) using a syringe while maintaining magnetic stirring. After an overnight reaction, TLC showed a UV absorbing compound R_f 0.52 (hexane-EtOAc 2:1) which chars with ninhydrin upon heating. Extraction (CH₂Cl₂- dil. NaHCO₃), drying (MgSO₄), evaporation and chromatography (gradient) hexane-EtOAc 3:1→2:1, gave **31** as colorless oil (20.3 g, 85%). Elution with CH₂Cl₂-MeOH 10:1 furnished monosilylated product **32** (0.12 g, 0.9%). Data for **31**: ¹H NMR (300 MHz, CDCl₃): δ = 7.65-7.32 (m, aromatic, 20 H), 3.71 (dd, J = 5.2, 9.8 Hz, 2 H), 3.62 (dd, J = 5.8, 9.8 Hz, 2 H), 3.00 (m of 5 lines, J = 5.6 Hz, 1 H), 1.51 (s, 2 H), 1.03 (s, 18 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 135.8, 133.7, 129.8, 127.9, 65.9, 54.6, 27.1, 19.5 ppm. HRMS (pos.): calcd. for C₃₅H₄₆NO₂Si₂ [M+H]⁺ 568.3061; found 568.3057. Data for **32**: ¹H NMR (300 MHz, DMSO-*d*₆): δ = 7.64-7.41 (m, aromatic, 10 H), 3.59 (dd, J = 6.9, 9.7 Hz, 1 H), 3.51 (dd, J = 5.8, 9.6 Hz, 1 H), 3.45 (dd, J = 5.3, 10.4 Hz, 1 H), 3.33 (dd, J = 6.0, 10.4 Hz, 1 H), 3.23 (br s, 2 H), 2.84 (m of 5 lines, J = 5.7 Hz, 1 H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆): δ = 135.1, 133.2, 129.8, 127.9, 66.1, 63.2, 54.3, 26.7, 18.9 ppm. HRMS (pos.): calcd. for C₁₉H₂₈NO₂Si [M+H]⁺ 330.1884; found 330.1894.

Diethoxyphosphonylmethyl tosylate (33a): Diethylhydroxymethyl phosphonate (Aldrich) (13.5 g, 78 mmol) was added to pyridine (30 ml) and CH₂Cl₂ (70 ml) and the resulting solution was cooled in an ice-bath under argon atmosphere. Tosyl chloride (14.9 g, 78 mmol) was added and the mixture was allowed to reach rt during 5 h and left at rt for 20 h. The mixture was partitioned between CH₂Cl₂ and water. The organic phase was washed with sat. NaHCO₃ followed by water, dried, filtered and the volatiles were evaporated. Xylenes were added and evaporated to remove any residual pyridine. Final drying on oil pump yielded crude product (23.4 g, 91%) which was used without any further purification. The yield obtained was higher than published.^[41] ¹H NMR (300 MHz, CDCl₃): δ = 7.80 (d, J = 8.3 Hz, 2 H), 7.37 (d, J = 8.0 Hz, 2 H), 4.23-4.09 (m unresolved, 6 H), 2.46 (s, 3 H), 1.31 (t, J = 7.1 Hz, 6 H) ppm. ¹³C NMR (75 MHz): δ = 145.6, 131.8, 130.1, 128.3, 63.5 (d, J_{CP} = 6.4 Hz), 61.4 (d, J_{CP} = 167.9 Hz), 21.7, 16.4 (d, J_{CP} = 5.7 Hz) ppm. ³¹P NMR (121 MHz, CDCl₃): δ = 15.2 ppm. Attempts to obtain diethylphosphonomethyloxysulphonoimidazole (to be used instead of triflate **33b** or tosylate **33a**) by direct reaction of diethylhydroxymethyl phosphonate with sulfuryldiimidazole^[44] and NaH, or with sulfuryl chloride and imidazole^[43], followed by addition of the amine **39** e.g. furnished no traces of the expected product **40** (see below).

Diethoxyphosphonylmethyl triflate (33b): Compound **33b** was prepared from diethoxyhydroxymethylphosphonate, trifluoromethylsulfonyl anhydride and 2,6-lutidine as published^[42] (an alternative is to use CF₃SO₂Cl and NaH^[47]) and was used as a crude product without any further purification (such as distillation^[42]). This material was most probably contaminated with bis(diethylphosphonomethyl) ether.^[47] Diethylhydroxymethyl phosphonate (5.2 g, 4.5 ml, 30.6 mmol) in dry CH₂Cl₂ (50 ml, freshly distilled from CaH₂) and 2,6-lutidine (dried by storage over KOH, 4.3 ml, 37.6 mmol) was cooled to ca -50 °C (external temp.)

under argon. To this solution was slowly added trifluoromethylsulfonic anhydride (10.1 g, 6.0 ml, 35.6 mmol) *via* a syringe during *ca* 10 min while maintaining magnetic stirring. After addition was complete, the temperature was increased slowly to *ca* 10 °C during 90 min, whereupon extraction was performed (CH₂Cl₂-dil. HCl). The organic phase was washed once with ice-cold H₂O, dried (MgSO₄), filtered, evaporated and finally dried on oil pump to furnish 9.5 g of red oil (theoretical yield is 9.2 g). This material was used as such immediately after preparation.

(S)-3-N-Diethylphosphonomethyl-1,2-isopropylidenoxypropyl amine (34): (S)-Amine **22** (1.06 g, 8.0 mmol), tosylate **33a** (3.7 g, 11.5 mmol) and powdered molecular sieves 4 Å (2 g) in dry DMF (25 ml) were stirred under argon at 80 °C during 20 h. TLC showed the product **34** R_f 0.28 (CH₂Cl₂-MeOH 20:1) revealed with ninhydrin. Unreacted amine remained at the application point. The solid material was filtered off, and extraction was performed (CH₂Cl₂-H₂O). The organic phase was dried (MgSO₄), and the solvent was evaporated. Chromatography in CH₂Cl₂-MeOH 10:1 furnished **34** (0.49 g, 21.5%) as colorless oil. For the NMR data see compound **40**. Prolongation of the reaction time and/or using of higher temperature did not improve the yield. *a*_D +2.4°, *c* 3.7, CHCl₃; HRMS (pos.): calcd. for C₁₁H₂₅NO₅P [M+H]⁺ 282.1465; found 282.1478.

6-Deoxy-6-N-diethylphosphonomethyl-1,2;3,4-di-O-isopropylidene-(D)-galactosamine (35): Tosylate **33a** (0.88 g, 2.78 mmol) and galactosylamine **26** (0.72 g, 2.78 mmol) in dry DMF (10 ml) and powdered mol. sieves 4 Å (3 g) were stirred under argon at 80 °C for 20 h. TLC showed a new product R_f 0.40 (CH₂Cl₂-MeOH 20:0.8). Unreacted **33a** and **26** were still present. The solid material was filtered out using a sintered glass, and the filtrate was extracted (CH₂Cl₂-H₂O). After usual work-up the residue was purified by chromatography in CH₂Cl₂-MeOH 20:0.8 to furnish **35** as an oil (0.42 g, 37%). ¹H NMR (300 MHz, CDCl₃): δ = 5.52 (d, *J* = 5.0 Hz, 1 H), 4.59 (dd, *J* = 2.3, 7.9 Hz, 1 H), 4.31 (dd, *J* = 2.4, 5.1 Hz, 1 H), 4.20-4.10 (m, 5 H), 3.89 (ddd, *J* = 1.7, 4.4, 8.1 Hz, 1 H), 3.03 (dd, *J* = 2.6, 12.5 Hz, 2 H), 2.97 (dd, *J* = 4.3, 7.6 Hz, 1 H), 2.85 (dd, *J* = 4.4, 12.7 Hz, 1 H), 1.94 (br s, exchangeable, 1 H), 1.52 (s, 3 H), 1.44 (s, 3 H), 1.36 (s, 3 H), 1.34 (s, 3 H), 1.33 (s, 3 H), 1.31 (s, 3 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 109.4, 108.6, 96.5, 72.0, 71.0, 70.7, 66.7, 62.2 (d, *J*_{CP} = 6.6 Hz), 50.8 (d, *J*_{CP} = 14.9 Hz), 45.1 (d, *J*_{CP} = 155.4 Hz), 26.2, 26.1, 25.1, 24.5, 16.6 (d, *J*_{CP} = 5.7 Hz) ppm. ³¹P NMR (121 MHz, CDCl₃): δ = 25.9 ppm. HRMS (pos.): calcd. for C₁₇H₃₃NO₈P [M+H]⁺ 410.1938; found 410.1935.

(Rac)-3-N-Diethylphosphonomethyl-1,2-isopropylidenoxypropyl amine (40): Tosylphosphonate **33a** (3.7 g, 7.6 mmol) was added to a solution of (rac)-3-amino-1,2-isopropylidenoxy propane **39** (Aldrich) (1.0 g, 7.6 mmol) in dry DMF (12 ml) followed by powdered 4 Å molecular sieves (2 g). The mixture was stirred under argon during 12 h at 80° C. TLC showed the product **40** R_f 0.28 (CH₂Cl₂-MeOH 20:1), revealed with ninhydrin. Unreacted amine remained at the application point. The solid material was filtered off, and extraction was performed (CH₂Cl₂-H₂O). The organic phase was dried (MgSO₄), and the solvent was evaporated. Chromatography in CH₂Cl₂-MeOH 10:1 furnished **40** as yellowish oil (0.86 g, 30.2%). Prolongation of the reaction time and/or using of higher temperature did not improve the yield. ¹H NMR (300 MHz, DMSO-*d*₆): δ = 4.12 (m of 5 lines, *J* = 6.0 Hz, 1 H), 4.02 (m of 5 lines, *J* = 7.3 Hz, 4 H), 3.96 (dd, *J* = 6.5, 8.0 Hz, 1 H), 3.60 (dd, *J* = 6.5, 8.0 Hz, 1 H), 3.31 (br s, exchangeable, 1 H), 2.94 (d, *J* = 11.4 Hz, 2 H), 2.71 (d, *J* = 2.1 Hz, 1 H), 2.69 (d, *J* = 1.7 Hz, 1 H), 1.31 (s, 3 H), 1.26 (s, 3 H), 1.23 (t, *J* = 7.1 Hz, 6 H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆): δ = 108.1, 74.8, 66.9, 61.2 (d, *J*_{CP} = 6.5 Hz),

52.7 (d, *J*_{CP} = 12.8 Hz), 44.3 (d, *J*_{CP} = 154.0 Hz), 26.7, 25.3, 16.3 (d, *J*_{CP} = 5.5 Hz) ppm. ³¹P NMR (121 MHz, DMSO-*d*₆): δ = 26.1 ppm and impurity (ca 6%) at 24.4 ppm. HRMS (pos.): calcd. for C₁₁H₂₅NO₅P [M+H]⁺ 282.1465; found 282.1462.

1,3-Di-*n*-butyldiphenylsilyloxy-2-(N-diethoxyphosphonomethyl)-propyl amine (36): Triflate **33b** (4.2 g, *ca* 14 mmol) was added to a solution of the amine **31** (7.9 g, 14 mmol) in dry THF (30 ml) under argon. The reaction mixture became blue. Some warming took place. After 8 h at r.t., the newly formed compound **36** showed R_f 0.31 (hexane-EtOAc 3:2). Extractive work-up (CH₂Cl₂-H₂O) and chromatography in hexane-EtOAc 3:2 furnished the product (5.65 g, 56.6%). When tosylate **33a** was used instead of **33b** (in DMF at 100°, overnight), **36** was isolated in 14% yield. ¹H NMR (300 MHz, CDCl₃): δ = 7.64-7.32 (m, aromatic, 20 H), 4.10 (m of 5 lines, *J* = 7.3 Hz, 4 H), 3.69 (apparent d, *J* = 5.7 Hz, 4 H), 2.91 (d, *J*_{HP} = 13.5 Hz, 2 H), 2.80 (m of 5 lines, *J* = 5.6 Hz, 1 H), 1.86 (br s, 1 H), 1.28 (t, *J* = 7.1 Hz, 6 H), 1.02 (s, 18H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 135.7, 133.6, 129.9, 127.9, 63.4, 62.4, 62.2 (d, *J*_{CP} = 6.8 Hz), 43.7 (d, *J*_{CP} = 156.3 Hz), 27.0, 19.4, 16.6 (d, *J*_{CP} = 5.6 Hz) ppm. ³¹P NMR (121 MHz, CDCl₃): δ = 26.0 ppm. HRMS (pos.): calcd. for C₄₀H₅₇NO₅PSi₂ [M+H]⁺ 718.3507; found 718.3517.

(Rac)-3-N-(diethylphosphonomethyl)amino-1-triphenylmethoxy-2-propanol (37) and (rac)-3-N-bis-(diethylphosphonomethyl)amino-1-O-triphenylmethoxy-2-propanol (38): *Via* triflate **33b**: The triflate **33b** (0.5 g, *ca* 1.6 mmol) was added to a solution of **6** in THF (15 ml) under argon atmosphere. The mixture warmed up and became blue. After 8 h at r.t., TLC showed the bis-phosphonate **38** R_f 0.55 and monophosphonate **37** R_f 0.47 (CH₂Cl₂-MeOH 20:1.3). Extractive work-up (CH₂Cl₂-H₂O) followed by chromatography in CH₂Cl₂-MeOH 20:1.1 furnished **38** (0.23 g, 22%) and **37** (0.14 g, 18%). *Via* tosylate **33a**: To a DMF (10 ml) solution of the amine **6** (0.81 g, 2.4 mmol) was added tosylate **33a** (0.84 g, 2.6 mmol) and this mixture was incubated at 100 °C under argon during 9 h. TLC profile was similar to that described above, but additionally minor byproducts were present, which made the purification difficult. Extractive work-up and chromatography furnished **37** as a syrup (0.34 g, 27%). The doubly phosphonylated compound **38** was not isolated. Data for **38**: ¹H NMR (300 MHz, CDCl₃): δ = 7.45-7.18 (m, two groups of signals, 15 H), 4.11 (m of 6 lines, *J* = 7.4 Hz, 8 H), 3.87-3.80 (m of 9 lines, 1 H), 3.30-3.15 (m, 5 H), 3.09-3.03 (m, 2 H), 5.89 (dd, *J* = 8.8, 13.5 Hz, 1 H), 1.30 (dt, *J* = 3.7, 7.0 Hz, 12 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 144.1, 128.8, 127.9, 127.1, 86.7, 68.8, 65.7, 62.3, 62.2, 62.2, 62.1, 62.1, 61.1, 61.1, 61.0, 52.1 (d, *J*_{CP} = 154.6 Hz), 16.7, 16.6, 16.6 ppm. ³¹P NMR (121 MHz, CDCl₃): δ = 25.5 ppm. HRMS (pos.): calcd. for C₃₂H₄₅NNaO₈P₂ [M+Na]⁺ 656.2513; found: 656.2504. Data for **37**: ¹H NMR (300 MHz, CDCl₃): δ = 7.44-7.19 (m, two groups of signals, 15 H), 4.10 (m of 5 lines, *J* = 7.3 Hz, 4 H), 3.86 (unresolved m of 5 lines, 1 H), 3.17 (dd, *J* = 5.8, 9.4 Hz, 1 H), 3.12 (dd, *J* = 5.1, 9.5 Hz, 1 H), 2.97 (apparent dd, *J* = 2.5, 12.3 Hz, 2 H), 2.90-2.70 (m, 3 H), 1.29 (t, *J* = 7.1 Hz, 6 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 144.0, 128.8, 128.0, 127.2, 86.8, 69.3, 66.1, 62.2 (d, *J*_{CP} = 6.6 Hz), 53.8 (d, *J*_{CP} = 13.6 Hz), 45.3 (d, *J*_{CP} = 153.3 Hz), 16.6 (d, *J*_{CP} = 6.0 Hz) ppm. ³¹P NMR (121 MHz, CDCl₃): δ = 26.0 ppm. HRMS (pos.): calcd. for C₂₇H₃₅NO₅P [M+H]⁺ 484.2247; found 484.2247.

Thymin-1-yl acetic acid (41): Compound **41** was obtained by direct alkylation of thymine with chloroacetic acid in alkaline medium in 46% yield, as described.^[45] ¹H NMR (300 MHz, DMSO-*d*₆): δ = 13.1 (br s, exchangeable, 1 H), 7.49 (unresolved q, *J* = 1.1 Hz, 1 H), 4.37 (s, 2 H), 1.75 (d, *J* = 1.1 Hz, 3 H) ppm. ¹³C

NMR (75 MHz): δ = 169.7, 164.34, 151.0, 141.8, 108.4, 48.4, 11.9 ppm.

(S)-3'-N-(Diethylphosphonomethyl)-1',2'-isopropylidenoxypropyl-thymine-1-yl acetamide (42): Coupling procedure as described below was performed using the amine **34** (0.42 g, 3.2 mmol), thymine acetic acid **41** (1.8 g, 9.6 mmol), DCC (2.0 g, 9.6 mmol) and HBT (1.0 g, 7.0 mmol) in dry DMF (20 ml) during 24 h. Work-up and chromatography yielded **42** (0.55 g, 82%). α_D -18.2°, c 3.1, CHCl₃. HRMS (pos.): calcd. for C₁₈H₃₁N₃O₈P [M+H]⁺ 448.1843; found: 448.1846.

((6'-N-6'-Deoxy-1',2';3',4'-di-O-isopropylidene-(D)-galactopyranosyl))-N-(diethylphosphonomethyl)-thymine-1-yl acetamide (43): To a magnetically stirred solution of the amine **35** (0.41 g, 1 mmol) and thymine acetic acid **41** (0.368 g, 2 mmol) in dry DMF (10 ml) was added dicyclohexylcarbodiimide DCC (0.412 g, 2 mmol) and hydroxybenzotriazole HBT (0.27 g, 2 mmol) under argon. The mixture became turbid in few seconds. After 36 h of stirring the solids were filtered off using a sintered glass. The filtrate was extracted using CH₂Cl₂ and aq NaHCO₃. The organic phase was dried (MgSO₄) and the volatiles were evaporated. The product which has R_f 0.38 in CH₂Cl₂-MeOH 20:1.4 was isolated by chromatography in the same system to furnish **43** (0.52 g, 92%). α_D -29.3° c 4.1 CHCl₃. The NMR spectra of **43** (just like of **43**, **44** and **45**) are very complex due to the couplings to ³¹P and the presence of *syn/anti* isomers around the amide bond. ¹H NMR (500 MHz, CDCl₃): δ = 9.42 (major) and 9.36 (minor) (2 x br s, ca 6:4 proportion rotamers, exchangeable, 1 H), 7.01 (minor) and 6.93 (major) (2 x d, rotamers, J = 1.1 Hz, 1 H), 5.53 (major) and 5.47 (minor) (2 x d, rotamers, J = 4.9 Hz, 1 H), 4.95 (major) and 4.86 (minor) (2 x d, J = 16.6 Hz, 1 H), 4.68 (d, J = 16.5 Hz) and 4.65 (dd, J = 2.2, 8.0 Hz) (1 H), 4.57 (dd, J = 2.2, 7.9 Hz) and 4.52 (d, J = 16.5 Hz) (1 H), 4.40 (t, J = 15.1 Hz, 0.5 H), 4.35 (major) and 4.28 (minor) (2 x dd, rotamers, J = 2.3, 4.9 Hz, 1 H), 4.23-3.99 (unresolved m, 6.5 H), 3.94-3.83 (unresolved m, 1.5 H), 3.71 (dd, J = 3.2, 15.7 Hz, 0.5 H), 3.46-3.36 (m, 1 H), 1.91 (major) and 1.89 (minor) (2 x s, rotamers, 3 H), 1.52-1.26 (m, 18 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 167.7, 167.6, 167.6, 164.5, 151.2 (major) and 151.1 (minor), 141.3 (minor) and 141.2 (major), 110.6 (major) and 110.5 (minor), 109.7 (major) and 109.6 (minor), 109.1 (minor) and 109.0 (major), 96.4 (major) and 96.3 (minor), 71.6 (minor) and 71.4 (major), 70.9, 70.5, 64.7, 63.1, 63.0, 62.97, 62.93, 62.8, 62.7, 62.6, 48.8, 48.3, 48.1, 47.5, 45.1 (minor, d, J_{CP} = 156.9 Hz), 40.4 (major, d, J_{CP} = 155.8 Hz), 26.2, 26.1, 26.0, 25.1, 24.9, 24.5, 24.4, 16.7, 16.6, 16.5, 16.4, 16.3, 12.5 (major) and 12.4 (minor) ppm. ³¹P NMR (121 MHz, CDCl₃): δ = 21.44 (major) and 21.13 (minor) ppm. HRMS (pos.): calcd. for C₂₄H₃₈N₃NaO₁₁P [M+Na]⁺ 598.2136; found 598.2143.

2-N-(Diethylphosphonomethyl)-1,3-bis-*t*-butyldiphenylsilyloxypropyl-thymine-1-yl acetamide (44): The amine **36** (3.9 g, 5.4 mmol) and thymine-1-yl-acetic acid **41** (3.0 g, 16.2 mmol) were condensed using DCC (3.3 g, 16.2 mmol) and HBT (1.4 g, 10 mmol) in dry DMF (60 ml) during 48 h under argon as described above. TLC showed the product **44** R_f 0.32 (hexane-EtOAc 1:4). After usual work-up and chromatography in hexane-EtOAc 1:4, 1.16 g, 24% of **44** was obtained. ¹H NMR (300 MHz, CDCl₃): δ = 8.97 (minor) and 8.88 (major) (1:2 proportion, 2 x br s, rotamers, exchangeable, 1 H), 7.59-7.34 (m, aromatic, 20 H), 6.89 (minor) and 6.50 (major) (2 x unresolved q, rotamers, J ca 1 Hz, 1 H), 4.68 (minor) and 4.29 (major) (2 x br s, rotamers, 2 H), 4.15-3.69 (unresolved m, 11 H), 1.88 (minor) and 1.79 (major) (2 x unresolved d, J ca 1 Hz, 3 H), 1.23 (minor) and 1.16 (major) (2 x t, rotamers, J = 7.1 Hz, 6 H), 1.03 (major) and 1.00 (minor) (2 x s, rotamers, 18 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 167.7,

164.5, 151.1 (minor) and 150.9 (major), 141.2 (minor) and 141.1 (major), 135.7, 135.6, 133.2, 133.1, 132.9, 132.8, 130.2, 130.1, 129.9, 128.1, 127.9, 110.5 (minor) and 110.3 (major), 63.0 (minor, d, J_{CP} = 7.4 Hz), 62.5 (major, d, J_{CP} = 6.5 Hz), 62.4 (minor) and 62.2 (major), 60.2, 48.6 (minor) and 48.2 (major), 39.1 (d, J_{CP} = 158.7 Hz), 27.0 (major) and 26.9 (minor), 19.3 (major) and 19.2 (minor), 16.6 (minor, d, J_{CP} = 5.3 Hz), 16.4 (major, d, J_{CP} = 6.0 Hz), 12.4 ppm. ³¹P NMR (121 MHz, CDCl₃): δ = 22.47 (major) and 20.89 (minor) ppm. HRMS (pos.): calcd. for C₄₇H₆₂N₃NaO₈PSi₂ [M+Na]⁺ 906.3705; found: 906.3716.

(Rac)-3-N-(Diethylphosphonomethyl)-1,2-O-isopropylidenoxypropyl-thymine-1-yl acetamide (45): To a magnetically stirred solution of the amine **40** (0.3 g, 1.1 mmol) and thymine-1-yl-acetic acid **41**^[45] (0.55 g, 3.0 mmol) in dry DMF (10 ml), was added dicyclohexylcarbodiimide (DCC, 0.62 g, 3.0 mmol) and 1-hydroxybenzotriazole (HBT, 0.27 g, 2.0 mmol) under argon. The mixture became turbid in few seconds. Stirring was continued overnight. TLC showed the product R_f 0.38 (CH₂Cl₂-MeOH 20:1.3). The solids were filtered off using a sintered glass. The filtrate was transferred to a separatory funnel charged with CH₂Cl₂ and aq NaHCO₃ and extraction was performed. The organic phase was dried (MgSO₄) and volatiles were evaporated. Chromatography of the residue (gradient CH₂Cl₂-MeOH 20:1.2→20:1.4) furnished **45** as a foam (0.43 g, 90.1%). ¹H NMR (300 MHz, CDCl₃): δ = 9.86 (br s, exchangeable, 1 H), 7.04 (major) and 7.00 (minor) (2 x br s, rotamers, proportion 3:2, 1 H), 4.88-3.67 (unresolved m, 13 H), 1.90 (s, 3 H), 1.47-1.27 (unresolved m, 12 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 167.7 (major) and 167.3 (major), 164.5 (major) and 164.4 (minor), 151.4 (major) and 151.3 (minor), 141.2 (major) and 141.1 (minor), 110.8 (major) and 110.5 (minor), 109.7, 75.4 (major) and 73.8 (minor), 67.1 (major) and 67.0 (minor), 63.2, 63.1, 63.0, 62.9, 62.9, 62.8, 62.7, 50.8 (minor) and 49.7 (major), 48.4 (major) and 48.1 (minor), 44.7 (major, d, J_{CP} = 157.2 Hz), 41.5 (minor, d, J_{CP} = 155.1 Hz), 26.8, 25.5 (major) and 25.3 (minor), 16.7 (major, d, J_{CP} = 5.4 Hz), 16.6 (minor, d, J_{CP} = 5.6 Hz), 12.5 (minor) and 12.4 (major) ppm. ³¹P NMR (121 MHz, CDCl₃): δ = 21.40 (minor) and 21.09 (major) ppm. HRMS (pos.): calcd. for C₁₈H₃₁N₃O₈P [M+H]⁺ 448.1843; found 448.1855.

((6'-N-6'-Deoxy-(D)-galactopyranosyl))-N-(diethylphosphonomethyl)-thymine-1-yl acetamide (46): 6'-N-6'-Deoxy-1,2;3,4-di-O-isopropylidene-(D)-galactopyranosyl-N-(diethylphosphonomethyl)-thymine-1-yl acetamide **43** (1.76 g, 3.06 mmol) was mixed with 80% aq. TFA (50 ml). After 1 h TLC showed consumption of all the starting material. After removal of all the volatiles *in vacuo* and drying, water was added to the residual oil, followed by Amberlite IRA 400 OH⁻ until neutrality. The resin was filtered and washed with water. The combined filtrates were evaporated and dried *in vacuo* to leave a crude residue which was then purified by column chromatography (CH₂Cl₂:MeOH 80:20, 1% EtN₃) to give compound **46** as a white foam (1.31 g, 86%). The NMR spectra of **46** is very complex due to the couplings to ³¹P, the presence of *syn/anti* isomers around the amide bond and α/β anomers at C1'. ¹H NMR (500 MHz, MeOD): δ = 7.34 (major), 7.32 (minor), 7.30 (major) and 7.27 (minor) (4 x d, J = 1.2 Hz, 1 H), 5.50 (major, d, J = 3.7 Hz) and 5.17 (minor, d, J = 2.4 Hz) and 5.14 (minor, d, J = 3.2 Hz) (1 H), 4.92-4.77 (unresolved m), 4.46 (d, J = 7.3 Hz, 0.5 H), 4.40-4.38 (minor) and 4.34-4.32 (major) (2 x m, 1 H), 4.25-4.00 (unresolved m, 6.5 H), 3.94-3.92 (unresolved m, 1 H), 3.85-3.74 (unresolved m, 3 H), 3.64-3.58 (unresolved m, 1 H), 3.54-3.43 (unresolved m, 1.5 H), 3.31-3.29 (m, 0.5 H), 3.20 (q, J = 7.4 Hz, 2 H), 1.86 (minor) and 1.85 (major) (2 x d, J = 1.1 Hz, 3 H), 1.37 (minor) and 1.30 (major) (2 x t, J = 7.2 Hz, 6 H) ppm. ¹³C NMR (125 MHz, MeOD): δ = 170.0, 169.8, 167.0, 166.9, 153.1, 153.0,

143.8, 143.7, 110.9, 110.8, 98.8, 98.7, 94.2, 94.1, 74.8, 74.7, 74.1, 73.6, 72.8, 71.5, 71.3, 71.0, 70.9, 70.8, 70.6, 70.2, 69.3, 68.4, 64.5, 64.23, 64.17, 64.1, 64.0, 53.6, 49.9, 49.8, 49.7, 47.8, 42.9, 42.3 (major, d, J_{CP} = 157.1 Hz), 42.3 (major, d, J_{CP} = 157.1 Hz) and 42.2 (minor, d, J_{CP} = 156.8 Hz), 16.83, 16.79, 16.75, 16.70, 16.6, 12.2, 12.1. ^{31}P NMR (121 MHz, MeOD): δ = 22.9, 22.8, 22.6 (major) and 21.8 ppm. HRMS (pos.): calcd. for $\text{C}_{18}\text{H}_{30}\text{N}_3\text{NaO}_{11}\text{P}$ $[\text{M}+\text{Na}]^+$ 518.1510; found 518.1516.

***N*-(Diethylphosphonomethyl)-*N*-[(*R*)-2,3-dihydroxypropyl]-thymine-1-yl acetamide (47):** To a stirred solution of (6'-*N*-6'-deoxy-(*D*)-galactopyranosyl)-*N*-(diethylphosphonomethyl)-thymine-1-yl acetamide **46** (0.30 g, 0.61 mmol) in absolute ethanol (8 ml) at 0 °C was added sodium periodate (0.52 g, 2.43 mmol) solubilized in a small volume of water (2.5 ml). The mixture turned immediately opaque. The reaction was completed after stirring for 1 h, it was then filtered through a pad of Celite washing with ethanol and the filtrate was concentrated *in vacuo* at 25 °C. The resulting white solid residue was then dried under reduced pressure and used in the following step without further purification. The crude residue was suspended in absolute ethanol and the mixture was cooled to 0 °C. Sodium borohydride (0.045 g, 1.19 mmol) was then added and the reaction mixture was allowed to warm to r.t. The stirring was continued for 3 h. The reaction mixture was then quenched with 10 % HOAc until pH = 7. All the volatiles were removed *in vacuo* to leave a crude product which was purified by column chromatography (CH_2Cl_2 :MeOH 9:1) to give compound **47** (0.11 g, 52%) as a white solid. A degradation fragment **49** was also isolated (0.016 g, 8%), while traces (< 1%) of fragment **48** were identified by mass spectroscopy [HRMS (neg.): calcd. for $\text{C}_{14}\text{H}_{23}\text{N}_3\text{O}_7\text{P}$ $[\text{M}-\text{H}]^-$ 376.1279; found 376.1266] but the compound was not fully characterized. Data for **47**: ^1H NMR (500 MHz, D_2O): δ = 7.38 (major) and 7.36 (minor) (2 x d, rotamers, J = 1.1 Hz, 1 H), 4.88 (ddd, J = 1.7, 17.0, 35.6 Hz, 2 H), 4.30-4.14 (unresolved m, 5 H), 4.09-4.07 (major) and 4.03-3.98 (minor) (2 x m, rotamers, 1 H), 3.91 (dd, J = 11.1, 16.0 Hz, 1 H), 3.70-3.51 (unresolved m, 4 H), 1.90 (s, 3 H), 1.38 (minor, t, J = 7.1 Hz) and 1.33 (major, dt, J = 1.5, 7.5 Hz) (rotamers, 6 H) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 169.0 (minor) and 168.8 (major), 166.7, 151.9 (minor) and 151.8 (major), 142.8 (major) and 142.7 (minor), 110.6 (minor) and 110.5 (major), 69.1 (minor) and 68.5 (major), 64.3 (minor, d, J_{CP} = 7.2 Hz) and 63.9 (major, d, J_{CP} = 6.7 Hz), 62.9 (minor) and 62.6 (major), 50.1 (major) and 50.0 (minor), 49.2 (minor) and 49.1 (major), 43.6 (minor, d, J_{CP} = 156.9 Hz) and 41.2 (major, d, J_{CP} = 155.7 Hz), 15.5 (minor, d, J_{CP} = 5.4 Hz) and 15.3 (major, d, J_{CP} = 5.7 Hz), 11.0 ppm. ^{31}P NMR (121 MHz, D_2O): δ = 24.0 (major) and 22.4 (minor) ppm. HRMS (pos.): calcd. for $\text{C}_{15}\text{H}_{26}\text{N}_3\text{NaO}_8\text{P}$ $[\text{M}+\text{Na}]^+$ 430.1349; found 430.1339. Data for **49**: ^1H NMR (MeOD, 300 MHz): δ = 7.35 (d, J = 1.2 Hz, 1 H), 4.42 (d, J = 1.4 Hz, 2 H), 4.18-4.08 (m, 4 H), 3.72 (d, J = 11.7 Hz, 2 H), 3.20 (q, J = 7.3 Hz, 1 H), 1.86 (d, J = 1.1 Hz, 3 H), 1.33-1.27 (m, 6 H) ppm. ^{13}C NMR (MeOD, 75 MHz): δ = 169.8, 165.2, 151.2, 141.8, 109.3, 62.5 (d, J_{CP} = 6.6 Hz), 49.0, 33.9 (d, J_{CP} = 158.3 Hz), 14.9 (d, J_{CP} = 5.9 Hz), 10.5 ppm. ^{31}P NMR (MeOD, 121 MHz): δ = 22.8 ppm. HRMS (pos.): calcd. for $\text{C}_{12}\text{H}_{21}\text{N}_3\text{O}_6\text{P}$ $[\text{M}+\text{H}]^+$ 334.1163; found 334.1163.

***N*-(*R*)-2,3-Dihydroxypropyl]-*N*-(phosphonomethyl)-thymine-1-yl acetamide (50):** To a solution of *N*-(diethylphosphonomethyl)-*N*-[(*R*)-2,3-dihydroxypropyl]-thymine-1-yl acetamide **47** (0.11 g, 0.27 mmol) in dry CH_2Cl_2 (5 ml) at 0 °C was added iodotrimethylsilane (0.15 ml, 1.08 mmol). The reaction mixture was stirred overnight at r.t. under an argon atmosphere. It was then quenched with a 1M TEAB solution and evaporated under reduced pressure. The crude residue was first purified by column chromatography (CH_2Cl_2 :MeOH:1M TEAB 5:2.5:0.5) and then by

HPLC using a Source 15Q anion exchange column (gradient 1M TEAB from 1 to 25% in 30 min) to afford the title compound **50** as a triethylammonium salt (76%). α_D +19.1°, c 0.52, DMSO-PBS; ^1H NMR (300 MHz, D_2O): δ = 7.41 (major, d, J = 1.1 Hz) and 7.35 (minor, br s) (rotamers, 1 H, H_6), 4.91 (br s, 2 H, COCH_2), 4.16-4.08 (minor) and 4.02-3.95 (major) (2 x m, rotamers, 1 H), 3.74 (minor) and 3.69 (major) (2 x d, rotamers, J = 4.2 Hz, 1 H), 3.67-3.47 (unresolved m, 5 H), 3.14 (q, J = 7.3 Hz, 12 H, $^+\text{N}(\text{CH}_2\text{CH}_3)_3$), 1.86 (s, 3 H, CH_3), 1.25 (t, J = 7.3 Hz, 18 H, $^+\text{N}(\text{CH}_2\text{CH}_3)_3$) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 169.6, 168.9, 154.3, 143.0, 110.4, 69.3 (major) and 68.4 (minor), 63.1 (major) and 63.0 (minor), 50.7, 49.5, 47.9 (d, J_{CP} = 136.9 Hz), 46.3, 11.3, 8.00 ppm. ^{31}P NMR (121 MHz, D_2O): δ = 12.6 (minor) and 12.0 (major) ppm. HRMS (neg.): calcd. for $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_8\text{P}$ $[\text{M}-\text{H}]^-$ 350.0759; found 350.0765.

***N*-(*S*)-2,3-Dihydroxypropyl]-*N*-(phosphonomethyl)-thymine-1-yl acetamide (51):** To a solution of (*S*)-*N*-(diethylphosphonomethyl)-3-*N*-(1,2-*O*-isopropylidene-*propyl*)-thymine-1-yl acetamide **42** (0.10 g, 0.22 mmol) in dry CH_2Cl_2 (5 ml) at 0 °C was added triethylamine (0.31 ml, 2.23 mmol), followed by iodotrimethylsilane (0.25 ml, 1.80 mmol). The reaction mixture was stirred overnight under an argon atmosphere. It was then quenched with a 1M TEAB solution and evaporated under reduced pressure. The crude residue was first purified by column chromatography (CH_2Cl_2 :MeOH:1M TEAB 5:2.5:0.5) and then by HPLC using a Source 15Q anion exchange column (gradient 1M TEAB from 1 to 25% in 30 min) to afford the title compound **51** as triethylammonium salt (78%). α_D -9.8°, c 0.88, DMSO-PBS; ^1H NMR (300 MHz, D_2O): δ = 7.41 (major) and 7.35 (minor) (2 x br s, rotamers, 1 H), 4.91 (br s, 2 H), 3.98-3.96 (m, 1 H), 3.74 (minor) and 3.69 (major) (2 x d, rotamers, J = 4.3 Hz, 1 H), 3.66-3.48 (m, unresolved, 5 H), 3.14 (q, J = 7.4 Hz, 12 H), 1.86 (s, 3 H), 1.24 (t, J = 7.4 Hz, 18 H) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 169.5, 169.2, 154.2, 143.4, 110.7, 69.6 (major) and 68.7 (minor), 63.4 (major) and 63.3 (minor), 51.0, 49.8, 48.1 (d, J_{CP} = 136.9 Hz), 46.6, 11.6, 8.31 ppm. ^{31}P NMR (121 MHz, D_2O): δ = 12.6 (minor) and 12.0 (major) ppm. HRMS (neg.): calcd. for $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_8\text{P}$ $[\text{M}-\text{H}]^-$ 350.0759; found 350.0760.

***N*-(*Rac*)-2,3-dihydroxypropyl]-*N*-(phosphonomethyl)-thymine-1-yl acetamide (52):** To a solution of (*rac*)-*N*-(diethylphosphonomethyl)-3-*N*-(1,2-*O*-isopropylidene-*propyl*)-thymine-1-yl acetamide **45** (0.40 g, 0.89 mmol) in dry CH_2Cl_2 (10 ml) at 0 °C was added triethylamine (1.24 ml, 8.89 mmol), followed by iodotrimethylsilane (0.76 ml, 5.35 mmol). The reaction mixture was stirred overnight under an argon atmosphere. It was then quenched with a 1M TEAB solution and evaporated under reduced pressure. The crude residue was first purified by column chromatography (CH_2Cl_2 :MeOH:1M TEAB 5:2.5:0.5) and then by HPLC using a Source 15Q anion exchange column (gradient 1M TEAB from 1 to 25% in 30 min) to afford the title compound **52** as triethylammonium salt (90%). ^1H NMR (300 MHz, D_2O): δ = 7.33 (major, d, J = 1.1 Hz) and 7.27 (minor, br s) (rotamers, 1H), 4.83 (br s, 2H), 4.08-3.99 (minor) and 3.92-3.86 (major) (2 x m, 1H), 3.66 (minor) and 3.61 (major) (2 x d, rotamers, J = 4.3 Hz, 1H), 3.54-3.38 (m, unresolved, 5H), 3.01 (q, J = 7.3 Hz, 12H), 1.78 (s, 3H), 1.16 (t, J = 7.3 Hz, 18H) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 169.5, 168.9, 154.2, 143.0, 110.4, 69.2 (major) and 68.4 (minor), 63.1 (major) and 62.9 (minor), 50.6, 49.5, 47.8 (d, J_{CP} = 136.8 Hz), 46.2, 11.3, 7.95 ppm. ^{31}P NMR (121 MHz, D_2O): δ = 12.6 (minor) and 12.0 (major) ppm. HRMS (neg.): calcd. for $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_8\text{P}$ $[\text{M}-\text{H}]^-$ 350.0759; found 350.0762.

***N*-(Diethylphosphonomethyl)-*N*-(1,3-dihydroxypropyl)-thymine-1-yl acetamide (53):** To a solution of 2-*N*-(diethylphosphonomethyl)-(1,3-bis-*t*-butyldiphenylsilyloxypropyl)-

thymine-1-yl acetamide **44** (0.20 g, 0.23 mmol) in dry DMF (2 ml) at 0 °C was added a solution of tris(dimethylamino)sulfonium difluorotrimethylsilicate (TAS-F, 0.15 g, 0.54 mmol) in dry DMF (2 ml). The reaction mixture was stirred at 0 °C for 2 h and then warmed to r.t.. It was then diluted with EtOAc and washed with pH 7 buffer. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over Na₂SO₄, filtered and concentrated *in vacuo*. The resulting crude residue was purified by column chromatography (CH₂Cl₂:MeOH 9:1) to give the title compound **53** (0.050 g, 54 %) as a white solid. ¹H NMR (300 MHz, MeOD): δ = 7.27 (major, d, *J* = 1.2 Hz) and 7.25 (minor, br s) (rotamers, 1 H), 4.80 (apparent d, *J* = 1.6 Hz, 2 H), 4.26-4.18 (minor) and 4.17-4.08 (major) (2 x m, rotamers, 4 H), 4.03-3.97 (m, 1 H), 3.87 (d, *J* = 12.0 Hz, 2 H), 3.70-3.68 (m, 2 H), 3.33-3.28 (m, 2 H), 1.86 (major) and 1.85 (minor) (2 x s, rotamers, 3 H), 1.38 and 1.30 (2 x t, rotamers, *J* = 7.1 Hz, 6 H) ppm. ¹³C NMR (75 MHz, MeOD): δ = 169.0, 165.3, 151.3, 142.0, 109.2, 62.5 (d, *J*_{CP} = 6.6 Hz), 61.1, 59.5 (minor) and 58.6 (major), 48.4, 37.7 (d, *J*_{CP} = 158.9 Hz), 14.9 (d, *J*_{CP} = 6.2 Hz), 10.5 ppm. ³¹P NMR (121 MHz, MeOD): δ = 25.6 (major) and 22.0 (minor) ppm. HRMS (pos.): calcd. for C₁₅H₂₆N₃NaO₈P [M+Na]⁺ 430.1350; found 430.1350.

***N*-(1,3-Dihydroxypropyl)-*N*-(phosphonomethyl)-thymine-1-yl acetamide (**54**):** To a solution of *N*-(diethylphosphonomethyl)-*N*-(1,3-dihydroxypropyl)-thymine-1-yl acetamide **53** (0.05 g, 0.12 mmol) in dry CH₂Cl₂ (3 ml) at 0 °C was added triethylamine (0.17 ml, 1.18 mmol), followed by iodotrimethylsilane (0.07 ml, 0.49 mmol). The reaction mixture was stirred overnight at r.t. under an argon atmosphere. It was then quenched with a 1M TEAB solution and evaporated under reduced pressure. The crude residue was first purified by column chromatography (CH₂Cl₂:MeOH:1M TEAB 5:2.5:0.5) and then by HPLC using a Source 15Q anion exchange column (gradient 1M TEAB from 1 to 25% in 30 min) to afford the title compound **54** as a triethylammonium salt (82%). ¹H NMR (300 MHz, D₂O): δ = 7.49 (major, d, *J* = 1.1 Hz) and 7.42 (minor, br s) (1 H, rotamers, H₆), 5.06 (br s, 2 H, COCH₂), 4.45-4.40 (m, 1 H, CH), 3.81 (dd, *J* = 12.3, 8.6 Hz, 2 H, CH₂), 3.67 (dd, *J* = 12.3, 4.7 Hz, 2 H, CH₂), 3.46 (d, *J* = 10.3 Hz, 2 H, POCH₂), 3.20 (q, *J* = 7.3 Hz, 12 H, ⁺N(CH₂CH₃)₃), 1.89 (minor) and 1.88 (major) (s, 3 H, CH₃), 1.27 (t, *J* = 7.3 Hz, 18 H, ⁺N(CH₂CH₃)₃) ppm. ¹³C NMR (75 MHz, D₂O): δ = 169.8, 167.1, 152.3, 143.3, 110.4, 61.4, 58.6 (major) and 57.8 (minor), 49.9, 46.3, 44.7 (d, *J*_{CP} = 134.9 Hz), 11.0, 7.92 ppm. ³¹P NMR (121 MHz, D₂O): δ = 13.7 (minor) and 12.3 (major) ppm. HRMS (neg.): calcd. for C₁₁H₁₇N₃O₈P [M-H]⁻, 350.0759; found 350.0766.

- [1] J. C. Chaput, H. Yu, S. Zhang, *Chem. Biol.* **2012**, *19*, 1360-1371.
- [2] K. U. Schoning, P. Scholz, S. Guntha, X. Wu, R. Krishnamurthy, A. Eschenmoser, *Science* **2000**, *290*, 1347-1351.
- [3] P. E. Nielsen, *Acc. Chem. Res.* **1999**, *32*, 624-630.
- [4] E. Meggers, L. Zhang, *Acc. Chem. Res.* **2010**, *43*, 1092-1102.
- [5] P. E. Nielsen, *Chem. Biodivers.* **2010**, *7*, 786-804.
- [6] P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science* **1991**, *254*, 1497-1500.
- [7] M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, P. E. Nielsen, *Nature* **1993**, *365*, 566-568.
- [8] A. Peyman, E. Uhlmann, K. Wagner, S. Augustin, G. Breipohl, D. W. Will, A. Schafer, H. Wallmeier, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2636-2638.
- [9] A. C. vanderLaan, R. Stromberg, J. H. vanBoom, E. Kuylyeheskiely, V. A. Efimov, O. G. Chakhmakhcheva, *Tetrahedron Lett.* **1996**, *37*, 7857-7860.
- [10] V. A. Efimov, M. V. Choob, A. L. Kalinkina, O. G. Chakhmakhcheva, R. Stromberg, A. C. VanDerLaan, N. J. Meeuwenoord, E. Kuylyeheskiely, J. H. VanBoom, *Collect. Czech. Chem. Commun.* **1996**, *61*, S262-S264.
- [11] V. A. Efimov, M. V. Choob, A. A. Buryakova, O. G. Chakhmakhcheva, *Nucleosides Nucleotides* **1998**, *17*, 1671-1679.
- [12] J. Kehler, U. Henriksen, H. Vejbjerg, O. Dahl, *Bioorg. Med. Chem.* **1998**, *6*, 315-322.
- [13] A. Peyman, E. Uhlmann, K. Wagner, S. Augustin, C. Weiser, D. W. Will, G. Breipohl, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2809-2812.
- [14] V. A. Efimov, M. V. Choob, A. A. Buryakova, A. L. Kalinkina, O. G. Chakhmakhcheva, *Nucleic Acids Res.* **1998**, *26*, 566-575.
- [15] V. A. Efimov, O. G. Chakhmakhcheva, E. Wickstrom, *Nucleos Nucleot Nucl* **2005**, *24*, 1853-1874.
- [16] M. Renders, G. Emmerechts, J. Rozenski, M. Krecmerova, A. Holy, P. Herdewijn, *Angew. Chem.* **2007**, *46*, 2501-2504.
- [17] A. V. Azhayev, M. L. Antopolsky, *Tetrahedron* **2001**, *57*, 4977-4986.
- [18] H. Ouyang, S. L. Morris-Natschke, K. S. Ishaq, P. Ward, D. Liu, S. Leonard, D. R. Thakker, *J. Med. Chem.* **2002**, *45*, 2857-2866.
- [19] B. Doboszewski, P. Herdewijn, *Tetrahedron Lett.* **2011**, *52*, 3853-3855.
- [20] S. David, S. Hanessian, *Tetrahedron* **1985**, *41*, 643-663.
- [21] J. P. Kutney, A. H. Ratcliffe, *Synth. Commun.* **1975**, *5*, 47-52.
- [22] K. C. K. Swamy, N. N. B. Kumar, E. Balaraman, K. V. P. P. Kumar, *Chem. Rev.* **2009**, *109*, 2551-2651.
- [23] J. M. Macdonald, H. T. Horsley, J. H. Ryan, S. Saubern, A. B. Holmes, *Org. Lett.* **2008**, *10*, 4227-4229.
- [24] D. Terakado, H. Koutaka, T. Oriyama, *Tetrahedron: Asymmetry* **2005**, *16*, 1157-1165.
- [25] Y. Gao, R. M. Hanson, J. M. Klunder, S. Y. Ko, H. Masamune, K. B. Sharpless, *J. Am. Chem. Soc.* **1987**, *109*, 5765-5780.
- [26] M. Pallavicini, E. Valoti, L. Villa, O. Piccolo, *Tetrahedron: Asymmetry* **1994**, *5*, 5-8.
- [27] W. L. Nelson, J. E. Wennerstrom, S. R. Sankar, *J. Org. Chem.* **1977**, *42*, 1006-1012.
- [28] E. A. Losey, M. D. Smith, M. Meng, M. D. Best, *Bioconj. Chem.* **2009**, *20*, 376-383.
- [29] T. Sato, T. Mizutani, Y. Okumura, T. Fujisawa, *Tetrahedron Lett.* **1989**, *30*, 3701-3702.
- [30] S. Nagarajan, B. Ganem, *J. Org. Chem.* **1987**, *52*, 5044-5046.
- [31] F. S. Gibson, M. S. Park, H. Rapoport, *J. Org. Chem.* **1994**, *59*, 7503-7507.
- [32] K. Danielmeier, E. Steckhan, *Tetrahedron: Asymmetry* **1995**, *6*, 1181-1190.
- [33] G. J. Wang, R. I. Hollingsworth, *J. Org. Chem.* **1999**, *64*, 1036-1038.
- [34] C. E. Ballou, H. O. L. Fischer, *J. Am. Chem. Soc.* **1954**, *76*, 3188-3193.
- [35] R. S. Tipson, *Methods Carbohydr. Chem.* **1963**, *2*, 246-250.
- [36] J. A. May, A. C. Sartorelli, *J. Med. Chem.* **1979**, *22*, 971-976.
- [37] W. A. Szarek, G. W. Hay, B. Doboszewski, *J. Chem. Soc., Chem. Commun.* **1985**, 663-664.
- [38] B. Doboszewski, G. W. Hay, W. A. Szarek, *Can. J. Chem.* **1987**, *65*, 412-419.
- [39] B. Doboszewski, P. R. da Silva, A. Y. Nazarenko, V. N. Nemykin, *Acta Cryst. E* **2010**, *66*, 3217-3218.
- [40] B. Streicher, B. Wunsch, *Carbohydr. Res.* **2003**, *338*, 2375-2385.

- [41] J. J. Bronson, L. M. Ferrara, H. G. Howell, P. R. Brodfuehrer, J. C. Martin, *Nucleosides Nucleotides* **1990**, 9, 745-769.
- [42] X. Creary, T. L. Underiner, *J. Org. Chem.* **1985**, 50, 2165-2170.
- [43] J. M. Vatele, S. Hanessian, *Tetrahedron* **1996**, 52, 10557-10568.
- [44] H. A. Staab, K. Wendel, *Liebigs Ann. Chem.* **1966**, 694, 86-90.
- [45] A. S. Jones, P. Lewis, S. F. Withers, *Tetrahedron* **1973**, 29, 2293-2296.
- [46] K. A. Scheidt, H. Chen, B. C. Follows, S. R. Chemler, D. S. Coffey, W. R. Roush, *J. Org. Chem.* **1998**, 63, 6436-6437.
- [47] D. P. Phillion, S. S. Andrew, *Tetrahedron Lett.* **1986**, 27, 1477-1480.

The C5 atom in 6-amino-6-deoxygalactopyranose was used as a source of chirality to obtain (*), whereas its enantiomer and achiral were conveniently synthesized starting from (S)-3-aminoglycerol and 2-amino-glycerol, respectively. The final compounds represent new monomeric precursors to backbone-modified nucleic acids of the pPNA-type.

Bogdan Doboszewski, Elisabetta Groaz, and Piet Herdewijn..... Page No. – Page No.

Synthesis of new phosphonoglycine backbone units for the development of unnatural phosphono peptide nucleic acids.

Keywords: Nucleotides/Phosponates/Phosphono peptide nucleic acid (pPNA)