Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 558–566
Carbohydrate RESEARCH

Potential sialyltransferase inhibitors based on neuraminyl substitution by hetaryl rings

Bini Mathew[†] and Richard R. Schmidt[*]

Fachbereich Chemie, Universität Konstanz, Fach M 725, D-78457 Konstanz, Germany

Received 20 June 2006; accepted 18 August 2006
Available online 20 September 2006

Dedicated to the memory of Professor Nikolay K. Kochetkov

Abstract—Replacement of the neuraminyl residue by a wide range of aryl rings in transition-state analogs of CMP-Neu5Ac led to readily accessible and potent inhibitors of α-(2→6)- and α-(2→3)-sialyltransferases. The synthesis of a series of potential sialyltransferase inhibitors in which the neuraminyl residue is replaced by hetaryl methylphosphonate residues (thiazole, benzothiazole, benzoxazole, benzothiophene and thiophene) is described in this paper.

Keywords: Sialyltransferases; Inhibitors; Transition-state analogs; Heterocycles; Phosphonates

1. Introduction

Sialic acids play a vital role in a variety of fundamental physiologically and pathologically important processes such as cell–cell adhesion, immune defences, tumour cell metastasis and inflammation.[1] Investigations have revealed a direct correlation between the number of sialic acid residues on the cell surface and the metastatic potential of tumour cells.[2] The hypersialylation, which has been observed on malign transformed cells, goes along with an enhanced sialyltransferase activity.[3] Therefore, inhibition of the transfer of sialic acids onto terminal positions of oligosaccharide chains is of great relevance and may lead to a decrease of metastases.

There are a family of sialyltransferases, which catalyze the transfer of sialic acids [essentially *N*-acetylneuraminic acid (Neu5Ac)] from cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) to nonreducing terminal positions of, for instance, cell-surface glycoproteins or glycosphingolipids. There is evidence that the transfer reaction proceeds through an S_N1-type mechanism involving partial dissociation of the CMP moiety and concomitant formation of a planar oxocarbenium ion in the transition state.[4,5] Hence, as previously shown, donor substrate transition-state analogs, as for instance **A** (Chart 1), having (i) a planar anomeric carbon, (ii) an increased distance between the anomeric carbon and the CMP leaving group and (iii) at least two negative charges close to the glycosylation site, exhibit high affinity to sialyltransferases.[6–10]

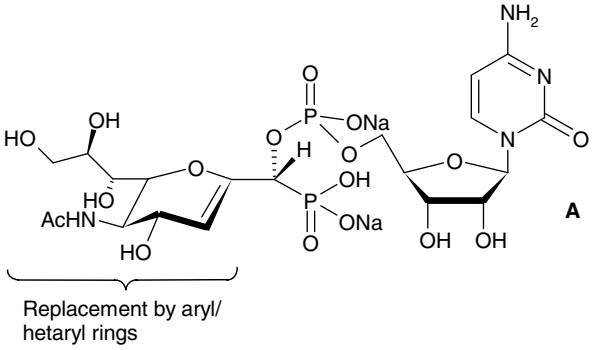


Chart 1. Sialyltransferase inhibitor **A**. Modification by aryl and hetaryl replacements.

* Corresponding author. Tel.: +49 7531 882538; fax: +49 7531 883135; e-mail: richard.schmidt@uni-konstanz.de
† Present address: Southern Research Institute, 2000, 9th Avenue South, Birmingham, AL 35205, USA.

0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.08.016

In previous studies, we have shown that the neuraminyl residue of transition-state analogous inhibitors can be replaced by a wide range of aryl methylphosphonate residues. Thus, readily accessible and highly potent inhibitors, for instance, of α-(2→6)-sialyltransferase from rat liver were obtained.[6,9,10] In continuation of our interest in this field, the synthesis of potential sialyltransferase inhibitors based on hetaryl rings such as thiazole, benzothiazole, benzoxazole, benzothiophene and thiophene was investigated. The structures of the target molecules **1a**–**e** are shown in Chart 2.

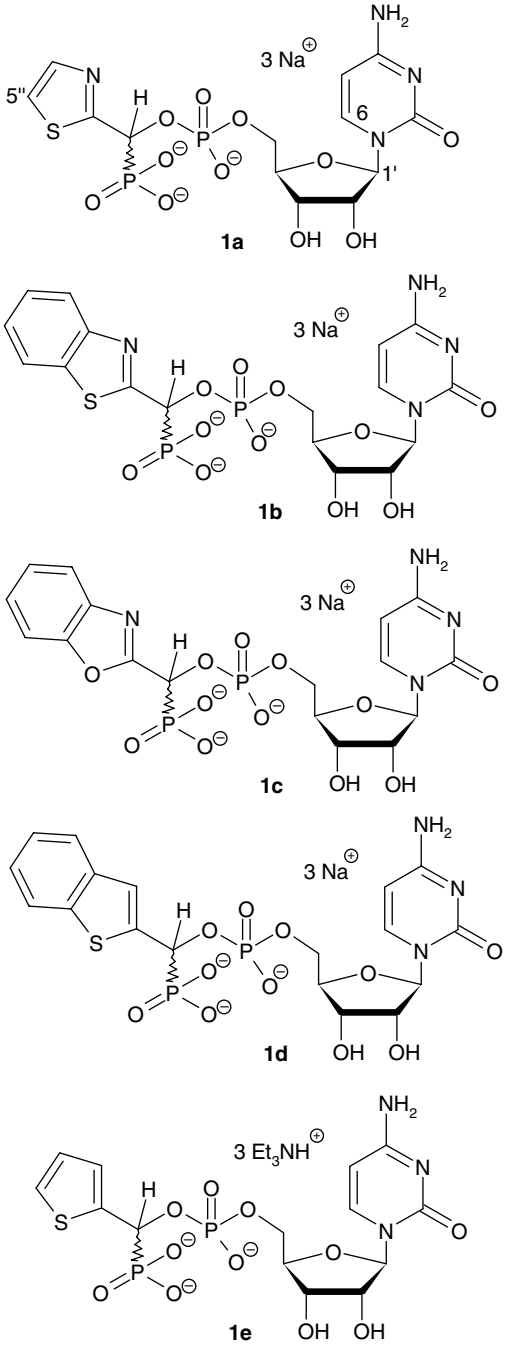
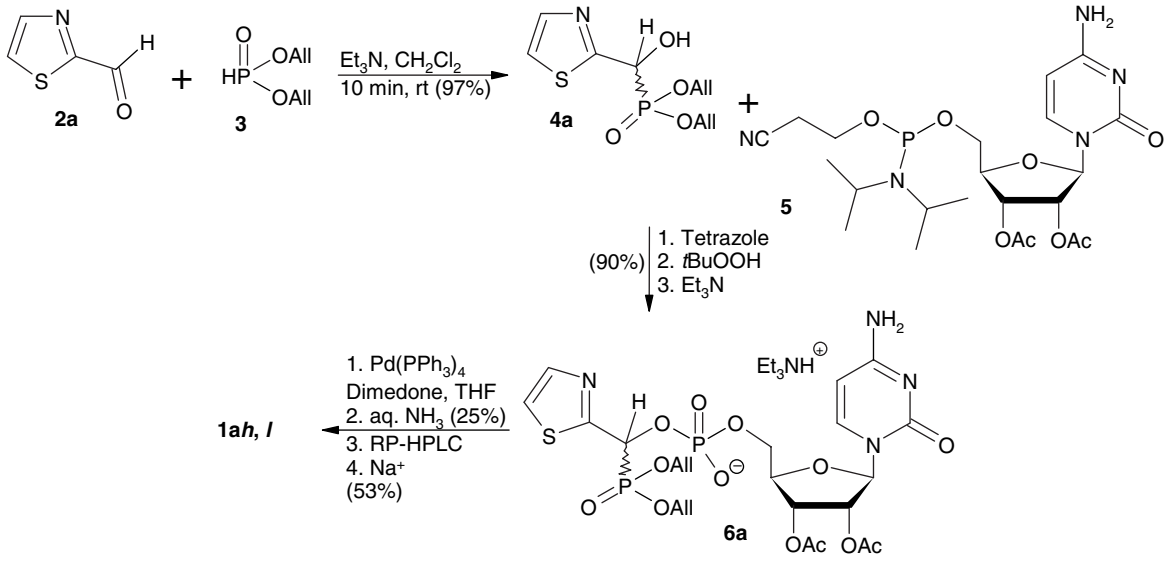


Chart 2. Target molecules **1a**–**e**.

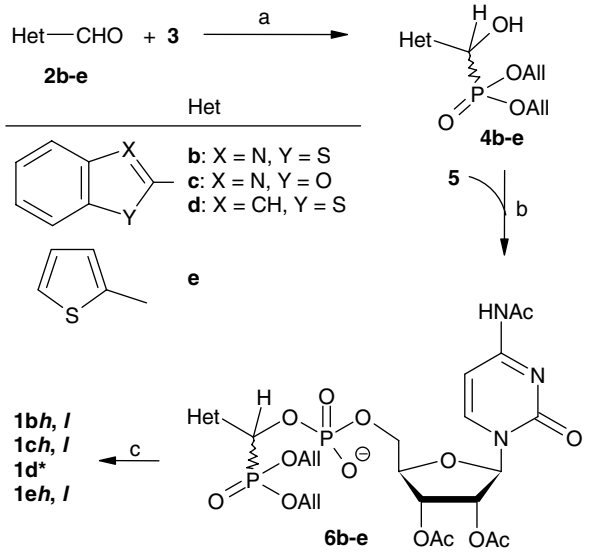
2. Results and discussion

Previous studies comparing the sialyltransferase inhibition activity of simple benzylcarboxylato and benzylphosphonato transition-state analogous inhibitors had shown that replacement of the carboxylate group by a phosphonate group increases the binding affinity by at least one order of magnitude.[6,9] Therefore, the corresponding α-hydroxy-hetarylmethylphosphonates were required as starting materials (Scheme 1). In order to investigate the reaction conditions for the synthesis of the target molecules **1a**–**e**, first commercially available thiazole-2-carbaldehyde (**2a**) was reacted with diallyl H-phosphonate **3**[11] in dichloromethane at room temperature in the presence of triethylamine as a base, thus affording an enantiomeric mixture of the desired α-hydroxyphosphonate **4a** in practically quantitative yield; the enantiomers were not separated at this stage. The allyl ester in the phosphonate moiety was selected in order to ease the release of the phosphonate group after synthesis of the protected target molecule. For the attachment of the CMP residue, the classical phosphite amidite methodology of Caruthers was chosen.[12] To this end, **4a** was treated with (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5-yloxy)-cyanoethoxy-diisopropylaminophosphane (**5**), prepared by the reaction of *N*-acetyl-2,3′-di-*O*-acetylcytidine with cyanoethoxy-bis(diisopropylamino)phosphane,[13] in the presence of tetrazole; following oxidation of the phosphite triester intermediate with *tert*-butylhydroperoxide and then cleavage of the cyanoethyl group with triethylamine furnished protected target molecule **6a** as diastereomeric mixture. O-Deallylation was readily carried out with catalytic amounts of $Pd(PPh_3)_4$ and dimedone as nucleophile. Aqueous ammonia was employed as reagent for N,O-deacetylation. Then, reversed-phase high-performance liquid chromatography (HPLC) with RP-18 as stationary phase and ethanol–water as eluents led to clean separation of the diastereoisomers, which, following previous experience,[6,8,9] may have different enzyme affinity. Finally, the products were converted to their sodium salts by ion exchange (IR 120, Na^+ form) to give the desired target molecules **1a*h*** and **1a*l*** (*h* and *l* are based on their difference in R_f value, *h*igh and *l*ow).

From **2b**,[14] **2c**,[15] **2d**[14] and commercially available **2e**, in a similar manner, α-hydroxyphosphonates **4b**–**e** were obtained in high yields (Scheme 2). Also reaction of these compounds with phosphite amidite **5** furnished the corresponding protected target molecules **6b**–**e** in good yields. Final deprotection and diastereoisomer separation as described for **6a** were readily performed for **6b** and **6c** affording **1b*h***, ***l*** and **1c*h***, ***l***, respectively, in good yields. Deprotection of **6d** led to **1d**; due to almost identical R_f values separation of the diastereoisomers was not possible. Deprotection of **6e** led to **1e*h*** and **1e*l***, which turned out to be quite labile under ion



Scheme 1. Synthesis of diastereoisomers **1a*h*** and **1a*l***.



Scheme 2. Synthesis of potential inhibitors **1b**–**e**. Reagents and conditions: (a) NEt_3, CH_2Cl_2 (90–95%); (b) 1. Tetrazole; 2. *t*-BuO_2H; NEt_3 (64–82%); (c) 1. $Pd(Ph_3)_4$, dimedone, THF; 2. aq NH_3; 3. RP-HPLC; 4. IE (Na^+) for **1b**–**d** (27–49%). *Diastereoisomers were not separated.

exchange conditions; therefore, they were isolated as tris(triethylammonium) salts.

All structures of new compounds could be confirmed by NMR and MS data. Activity testing of **1a**–**e** as inhibitors of α-(2→6)-sialyltransferase is underway.

In summary, the synthesis of transition-state analogs of the donor substrate CMP-Neu5Ac, in which the neuraminyl residue is replaced by a hetarylmethylphosphonate residue, could be performed in a few steps by addition of allyl H-phosphonate to hetaryl aldehydes, reaction of the resulting hydroxyphosphonates with cytidin-5′-yl phosphite amidites, oxidation to the phosphate and then deprotection. This way, the usefulness of this methodology is exhibited in the preparation of new potential inhibitors of sialyltransferases.

3. Experimental

3.1. General

The solvents were purified according to the standard procedures. Melting points are reported in degrees Celsius (uncorrected). NMR measurements were recorded at 22 °C on a Bruker AC 250 Cryospec, Bruker DRX 600, or a JEOL JNM−GX 400. Tetramethylsilane (TMS) or the resonance of the deuterated solvent was used as an internal standard; solvents: $CDCl_3$, $\delta = 7.24$; D_2O, $\delta = 4.63$; MeOH-d_4, $\delta = 3.305$. For ^{31}P NMR phosphoric acid was used as an external standard; ^{13}C NMR spectra were broadband ^1H decoupled. Matrix-assisted laser desorption ionisation mass spectra (MALDI-MS) were recorded on a Kratos Kompact Maldi 2, and 2,5-dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxy cinnamic acid (CHCA) was used as a matrix. Thin-layer chromatography was performed on silica gel plastic plates 60 F_{254} (E. Merck) or glass plates RP-18 (E. Merck); the compounds were visualised by treatment with a soln of $(NH_4)_6Mo_7O_{24}{\cdot}4\ H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% H_2SO_4 (400 mL). Flash chromatography was performed on silica gel (J. T. Baker, particle size 40 μm) at a pressure of 0.3–0.4 bar. Preparative HPLC was performed with a Shimadzu LC8A preparative pump and a Rainin Dynamax UV 1 detector at 260 nm. The columns used were (A) Eurospher 100-C18 (Knauer, 7 μm, 250 × 16 mm), (B)

Eurospher 100-C18 (Knauer, 7 μm, 250 × 20 mm), (C) LiChrospher 100 RP18 (E. Merck, 7 μm, 250 × 25 mm). Mixtures of MeCN and 0.05 M triethylammonium bicarbonate (TEAB) (pH 7.2–7.5) were used as mobile phase.

3.2. Diallyl hydroxy-(thiazol-2-yl)methylphosphonate (4a)

Thiazole-2-carbaldehyde **2a** (100 mg, 0.88 mmol) and diallyl H-phosphonate **3** (215 mg, 1.33 mmol) were dissolved in dry CH_2Cl_2 (5 mL) and Et_3N (0.2 mL) was added to the reaction mixture. The soln was stirred at rt for 10 min. The solvent was evaporated and flash column chromatography of the residue (20% acetone in toluene) afforded **4a** (235 mg, 97%) as a yellow viscous liquid. $R_f = 0.31$ (40% acetone in toluene). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.66 (d, 1H, $^3J_{5,4}$ 2.7 Hz, 5-H), 7.28 (d, 1H, $^3J_{4,5}$ 2.7 Hz, 4-H), 6.22 (br s, 1H, OH), 5.81–5.78 (m, 2H, allyl CH), 5.45 (d, 1H, $^2J_{1',P}$ 12.9 Hz, 1′-H), 5.24–5.08 (m, 4H, allyl CH_2), 4.50–4.49 (m, 4H, allyl CH_2). ^{13}C NMR (63 MHz, $CDCl_3$): δ 168.57 (2-C), 142.65, 133.05, 132.96, 120.50, 118.52, 118.44, 70.77, 68.42 (m, 1′-C). ^{31}P NMR (243 MHz, $CDCl_3$): δ 20.31 (s, PO_3). MALDIMS (positive mode, DHB-matrix): m/z 276.0 $[M+H]^+$.

3.3. Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetyl-cytidin-5′-yl)-[(diallylphosphonato)-(thiazol-2-yl)-methyl]-phosphate (6a)

Alcohol **4a** (110 mg, 0.4 mmol) and cytidine phosphoramidate **5** (341 mg, 0.6 mmol) were co-evaporated with dry CH_2Cl_2 and dried under diminished pressure for 1 h. Then the combined reactants were dissolved in dry CH_2Cl_2 (5 mL) and tetrazole (56 mg, 0.8 mmol) added to the reaction mixture. After stirring for 3 h at rt, *tert*-butylhydroperoxide (0.11 mL, 0.6 mmol) was added under cooling. After stirring for 2 h, Et_3N (2 mL) was added and the reaction mixture was stirred overnight. The solvent was evaporated and the resulting residue purified by flash column chromatography (20% MeOH in EtOAc) to afford **6a** (290 mg, 90%) as a pale yellow coloured lyophilisate. $R_f = 0.78$ (50% MeOH in EtOAc). ^{1}H NMR (250 MHz, CD_3OD): δ 8.35 (d, 0.5H, $^3J_{6,5}$ 7.5 Hz, 6-H), 8.29 (d, 0.5H, $^3J_{5,6}$ 7.5 Hz, 6-H), 7.79–7.45 (m, 3H, 5-H, 4″-H, 5″-H), 6.13–5.90 (m, 4H, 1″-H, 1′-H, allyl CH), 5.38–5.17 (m, 6H, 2′-H, 3′-H, allyl CH_2), 4.68–4.09 (m, 7H, 5′$_{a,b}$-H, 4′-H, allyl CH_2), 3.14 (q, 6H, J 7.3 Hz, –N–CH_2–CH_3), 2.17/2.16 (2s, 3H, –$OCOCH_3$), 2.08/2.07 (2s, 3H, –$OCOCH_3$), 2.04/2.03 (2s, 3H, HN$COCH_3$), 1.29 (t, 9H, J 7.3 Hz, –N–CH_2–CH_3). ^{13}C NMR (63 MHz, CD_3OD): δ 172.78, 171.13, 170.89, 166.80, 164.50, 157.80, 146.42, 143.51, 133.95, 122.57, 118.68, 98.86, 89.47, 83.10, 75.30, 74.39, 71.96, 71.84, 69.57 (m, 1″-C), 65.77, 47.54, 24.68, 20.54, 20.35, 19.35, 9.20. ^{31}P NMR (243 MHz, CD_3OD): δ 17.63 (combination of two d, $^3J_{P,P}$ 30.2 Hz, PO_3), 0.014 (combination of two d, $^3J_{P,P}$ 30.2 Hz, PO_4). MALDIMS (positive mode, CHCA-matrix): m/z 707.3 $[M-Et_3N+H]^+$, 729.3 $[M-Et_3N+Na]^+$, 745.3 $[M-Et_3N+K]^+$.

3.4. Trisodium cytidin-5′-yl-[phosphonato-(thiazol-2-yl)-methyl]-phosphate (1a*h*, *l*)

A soln of **6a** (200 mg, 0.25 mmol) in dry THF (20 mL) was treated with $Pd(PPh_3)_4$ (50 mg) and dimedone (173 mg, 1.24 mmol) at rt for 12 h. The solvent was evaporated and dimedone was removed from the reaction mixture by RP-18 chromatography (1:3 EtOH–water). After lyophilisation from water, it was dissolved in aq ammonia (4 mL) and stirred for 12 h. Again after lyophilisation from water, a mixture of diastereomers was separated by RP-18 HPLC (0.05 M TEAB) and finally lyophilised from water. The products were converted to their sodium salts by IR 120 (Na^+) and lyophilised to yield **1a*h*** (40 mg, 28%) and **1a*l*** (35 mg, 25%) as pale yellow solids.

3.4.1. Compound 1a*h*. HPLC: Prep. RP-18, Column A (flow 10 mL per min, 0.05 M triethylammonium bicarbonate buffer, 1% MeCN, $\chi = 260$ nm), $t_R = 17.5$ min. ^{1}H NMR (600 MHz, D_2O): δ 7.66 (d, 1H, $^3J_{6,5}$ 7.6 Hz, 6-H), 7.57 (d, 1H, $^3J_{5'',4''}$ 3.0 Hz, 5″-H), 7.40 (d, 1H, $^3J_{4'',5''}$ 3.0 Hz, 4″-H), 5.94 (d, 1H, $^3J_{5,6}$ 7.6 Hz, 5-H), 5.78 (d, 1H, $^3J_{1',2'}$ 4.8 Hz, 1′-H), 5.30 (dd, 1H, $^2J_{1'',P}$ 13.6 Hz, $^3J_{1'',P}$ 9.9 Hz, 1″-H), 4.00 (dd, 1H, $^3J_{2',1'}$ 4.8 Hz, $^3J_{2',3'}$ 4.9 Hz, 2′-H), 3.93–3.88 (m, 2H, 5′$_a$-H, 4′-H), 3.74 (dd, 1H, $J_{3',2'}$ 4.9 Hz, $^3J_{3',4'}$ 5.8 Hz, 3′-H), 3.67–3.65 (m, 1H, 5′$_b$-H). ^{13}C NMR (150 MHz, D_2O): δ 169.37 (2″-C), 165.48 (2-C), 157.12 (4-C), 140.72 (6-C), 140.32 (5″-C), 120.38 (4″-C), 96.09 (5-C), 88.31 (1′-C), 82.28 ($^3J_{4',P}$ 8.7 Hz, 4′-C), 74.33 ($^1J_{1'',P}$ 159.3 Hz, $^2J_{1'',P}$ 9.0 Hz, 1″-C), 73.57 (2′-C), 68.83 (3′-C), 64.06 (5′-C). ^{31}P NMR (243 MHz, D_2O): δ 11.66 (d, $^3J_{P,P}$ 30.1 Hz, PO_3), 2.15 (d, $^3J_{P,P}$ 30.1 Hz, PO_4). MALDIMS (negative mode, CHCA-matrix): m/z 567.0 $[M+H]^-$, 545.0 $[M-Na+2H]^-$, 523.0 $[M-2Na+3H]^-$, 500.9 $[M-3Na+4H]^-$.

3.4.2. Compound 1a*l*. HPLC: Prep. RP-18, Column A (flow 10 mL per min, 0.05 M triethylammonium bicarbonate buffer, 1% MeCN, $\chi = 260$ nm), $t_R = 20.2$ min. ^{1}H NMR (600 MHz, D_2O): δ 7.59 (d, 1H, $^3J_{6,5}$ 7.6 Hz, 6-H), 7.51 (d, 1H, $^3J_{5'',4''}$ 3.1 Hz, 5″-H), 7.34 (d, 1H, $^3J_{4'',5''}$ 3.1 Hz, 4″-H), 5.94 (d, 1H, $^3J_{5,6}$ 7.6 Hz, 5-H), 5.72 (d, 1H, $^3J_{1',2'}$ 3.6 Hz, 1′-H), 5.27 (dd, 1H, $^2J_{1'',P}$ 13.5 Hz, $^3J_{1'',P}$ 10.0 Hz, 1″-H), 3.95–3.84 (m, 5H, 2′-H, 3′-H, 4′-H, 5′$_{ab}$-H). ^{13}C NMR (150 MHz, D_2O): δ 169.45 (2″-C), 165.18 (2-C), 156.75 (4-C), 140.38 (6-C), 139.84 (5″-C), 119.88 (4″-C), 95.71 (5-C), 88.44 (1′-C), 81.65 ($^3J_{4',P}$ 9.0 Hz, 4′-C), 74.30

($^1J_{1'',P}$ 148.0 Hz, $^2J_{1'',P}$ 9.0 Hz, 1″-C), 73.52 (2′-C), 67.60 (3′-C), 62.93 (5′-C). ^{31}P NMR (243 MHz, D_2O): δ 11.47 (d, $^3J_{P,P}$ 29.7 Hz, PO_3), 2.15 (d, $^3J_{P,P}$ 29.7 Hz, PO_4). MALDIMS (negative mode, CHCA-matrix): m/z 566.9 $[M+H]^-$, 544.9 $[M-Na+2H]^-$, 522.9 $[M-2Na+3H]^-$, 500.9 $[M-3Na+4H]^-$.

3.5. Diallyl (benzothiazol-2-yl)-hydroxymethylphosphonate (4b)

Benzothiazole-2-carbaldehyde **2b**[14] (1.35 g, 8.28 mmol) and diallyl H-phosphonate **3** (2.01 g, 12.42 mmol) were dissolved in dry CH_2Cl_2 (15 mL) and Et_3N (0.3 mL) was added to the reaction mixture. The soln was stirred at rt for 30 min. The solvent was evaporated and flash column chromatography of the residue (20% acetone in toluene) afforded **4b** (2.55 g, 95%) as a pale yellow solid. Mp 40 °C. $R_f = 0.33$ (40% acetone in toluene). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.00 (d, 1H, J 8.0 Hz, ArH), 7.86 (d, J 7.5 Hz, ArH), 7.47–7.35 (m, 2H, ArH), 5.93–5.81 (m, 2H, allyl CH), 5.60 (d, 1H, $^2J_{1',P}$ 13.6 Hz, 1′-H), 5.33–5.13 (m, 4H, allyl CH_2), 4.63–4.56 (m, 4H, allyl CH_2). ^{13}C NMR (63 MHz, $CDCl_3$): δ 169.24, 153.06, 135.66, 132.99, 132.95, 132.90, 126.42, 125.54, 123.50, 122.08, 118.69, 71.48, 68.88, 68.77 (m, 1′-C). ^{31}P NMR (243 MHz, $CDCl_3$): δ 19.73 (s, PO_3). MALDIMS (positive mode, DHB-matrix): m/z 326.3 $[M+H]^+$.

3.6. Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(diallylphosphonato)-(benzothiazol-2-yl)-methyl]-phosphate (6b)

Alcohol **4b** (500 mg, 1.54 mmol) and cytidine phosphoramidate **5** (1.31 g, 2.31 mmol) were co-evaporated with dry CH_2Cl_2 and dried under diminished pressure for 1 h. Then the combined reactants were dissolved in dry CH_2Cl_2 (10 mL) and tetrazole (216 mg, 3.08 mmol) was added to the reaction mixture. After stirring for 3 h at rt, *tert*-butylhydroperoxide (0.42 mL, 2.31 mmol) was added under cooling. After stirring for 2 h, Et_3N (3 mL) was added and the reaction mixture stirred overnight. The solvent was evaporated and the residue purified by flash column chromatography (20% MeOH in EtOAc) to afford **6b** (920 mg, 70%) as a pale yellow lyophilisate. $R_f = 0.70$ (50% MeOH in EtOAc). ^{1}H NMR (250 MHz, CD_3OD): δ 8.33 (d, 0.5H, $^3J_{5,6}$ 7.6 Hz, 6-H), 8.25 (d, 0.5H, $^3J_{5,6}$ 7.6 Hz, 6-H), 7.99–7.92 (m, 2H, Ar–H), 7.50–7.38 (m, 3H, 5-H, Ar–H), 6.11–5.88 (m, 4H, 1″-H, 1′-H, allyl CH), 5.49–5.15 (m, 6H, 2′-H, 3′-H, allyl CH_2), 4.73–4.19 (m, 7H, $5'_{a,b}$-H, 4′-H, allyl CH_2), 3.11 (q, 6H, J 7.3 Hz, –N–CH_2–CH_3), 2.19/2.18 (2s, 3H, –OCOCH_3), 2.08/2.09 (2s, 3H, –OCOCH_3), 2.03/2.02 (2s, 3H, HNCOCH_3), 1.44 (t, 9H, J 7.3 Hz, –N–CH_2–CH_3). ^{13}C NMR (63 MHz, CD_3OD): δ 172.74, 171.06, 170.84, 168.45, 168.19, 164.44, 157.69, 157.64, 153.96, 153.89, 146.30, 136.55, 133.95, 129.98, 127.50, 126.83, 124.14, 123.11, 118.87, 98.78, 89.76, 89.60, 83.00, 75.29, 75.18, 71.85, 71.61 (m, 1″-C), 65.93, 47.49, 24.90, 20.63, 20.51, 19.41, 9.27. ^{31}P NMR (243 MHz, CD_3OD): δ 17.21 (d, $^3J_{P,P}$ 30.6 Hz, PO_3), 0.12 (combination of two d, $^3J_{P,P}$ 30.6 Hz, PO_4). MALDIMS (positive mode, CHCA-matrix): m/z 757.3 $[M-Et_3N+H]^+$, 779.3 $[M-Et_3N+Na]^+$, 795.3 $[M-Et_3N+K]^+$.

3.7. Trisodium cytidin-5′-yl-[(benzothiazol-2-yl)-phosphonatomethyl]-phosphate (1b*h*, *l*)

A soln of **6b** (180 mg, 0.21 mmol) in dry THF (20 mL) was treated with $Pd(PPh_3)_4$ (50 mg) and dimedone (147 mg, 1.05 mmol) at rt for 12 h. The solvent was evaporated and dimedone was removed from the reaction mixture by RP-18 chromatography (ethanol/water, 1:3). After lyophilisation from water, the residue was dissolved in aq ammonia (4 mL) and stirred for 12 h. After lyophilisation from water, the mixture of diastereomers was separated by RP-18 HPLC (0.05 M TEAB) and finally lyophilised from water. The products were converted to their sodium salts by IR 120 (Na^+) and lyophilised to yield **1b*h*** (30 mg, 23%) and **1b*l*** (27 mg, 21%) as pale yellow solids.

3.7.1. Compound 1b*h*. HPLC: Prep. RP-18, Column A (flow 10 mL per min, 0.05 M triethylammonium bicarbonate buffer, 2% MeCN, $\chi = 260$ nm), $t_R = 22.5$ min. ^{1}H NMR (600 MHz, D_2O): δ 7.71 (d, 1H, $^3J_{5'',6''}$ 8.0 Hz, 5″-H), 7.68 (d, 1H, $^3J_{8'',7''}$ 8.1 Hz, 8″-H), 7.30 (t, 1H, J 7.8 Hz, 6″-H), 7.21 (t, 1H, J 7.6 Hz, 7″-H), 7.16 (d, 1H, $^3J_{6,5}$ 7.5 Hz, 6-H), 5.66 (d, 1H, $^3J_{5,6}$ 7.5 Hz, 5-H), 5.39 (dd, 1H, $^2J_{1'',P}$ 14.0, $^3J_{1'',P}$ 10.0 Hz, 1″-H), 5.32 (d, 1H, $^3J_{1',2'}$ 2.5 Hz, 1′-H), 4.11 (bd, 1H, J 11.7 Hz, $5'_a$-H), 3.91–3.87 (m, 2H, $5'_b$-H, 4′-H), 3.62 (dd, 1H, $^3J_{3',4'}$ 6.8, $^3J_{3',2'}$ 5.0 Hz, 3′-H), 3.48 (dd, 1H, $^3J_{2',3'}$ 5.0, $^3J_{2',1'}$ 2.5 Hz, 2′-H). ^{13}C NMR (150 MHz, D_2O): δ 171.07 (2″-C), 165.03 (2-C), 156.14 (4-C), 150.42 (4″-C), 139.86 (6-C), 134.26 (9″-C), 125.62 (6″-C), 124.81 (7″-C), 121.31 (5″-C), 121.19 (8″-C), 95.60 (5-C), 88.97 (1′-C), 81.02 ($^3J_{4',P}$ 10.0 Hz, 4′-C), 75.38 ($^1J_{1'',P}$ 145.7, $^2J_{1'',P}$ 9.1 Hz, 1″-C), 73.75 (2′-C), 66.97 (3′-C), 62.52 (5′-C). ^{31}P NMR (243 MHz, D_2O): δ 11.11 (d, $^3J_{P,P}$ 29.5 Hz, PO_3), 2.69 (d, $^3J_{P,P}$ 29.5 Hz, PO_4). MALDIMS (negative mode, CHCA-matrix): m/z 617.0 $[M+H]^-$, 595.0 $[M-Na+2H]^-$, 573.0 $[M-2Na+3H]^-$, 551.1 $[M-3Na+4H]^-$.

3.7.2. Compound 1b*l*. HPLC: Prep. RP-18, Column A (flow 10 mL per min, 0.05 M triethylammonium bicarbonate buffer, 2% MeCN, $\chi = 260$ nm), $t_R = 25.8$ min. ^{1}H NMR (600 MHz, D_2O): δ 7.77 (d, 1H, $^3J_{5'',6''}$ 8.0 Hz, 5″-H), 7.73 (d, 1H, $^3J_{8'',7''}$ 8.1 Hz, 8″-H), 7.37–7.32 (m, 2H, 6″-H, 6-H), 7.24 (t, 1H, J 7.6 Hz,

7″-H), 5.75 (d, 1H, $^3J_{5,6}$ 7.5 Hz, 5-H), 5.43 (d, 1H, $^3J_{1',2'}$ 3.5 Hz, 1′-H), 5.34 (dd, 1H, $^2J_{1'',P}$ 14.3, $^3J_{1'',P}$ 9.7 Hz, 1″-H), 3.97 (bd, 1H, J 12.6 Hz, $5'_a$-H), 3.83–3.79 (m, 2H, $5'_b$-H, 4′-H), 3.69–3.59 (m, 2H, 2′-H, 3′-H). ^{13}C NMR (150 MHz, D_2O): δ 172.00 (2″-C), 165.36 (2-C), 156.71 (4-C), 150.54 (4″-C), 140.15 (6-C), 134.25 (9″-C), 125.58 (6″-C), 124.70 (7″-C), 121.42 (5″-C), 121.15 (8″-C), 95.68 (5-C), 88.78 (1′-C), 81.58 ($^3J_{4',P}$ 8.7 Hz, 4′-C), 75.64 ($^1J_{1'',P}$ 151.2 Hz, $^2J_{1'',P}$ 9.9 Hz, 1″-C), 73.74 (2′-C), 68.00 (3′-C), 63.85 (5′-C). ^{31}P NMR (243 MHz, D_2O): δ 10.96 (d, $^3J_{P,P}$ 29.3 Hz, PO_3), 2.64 (d, $^3J_{P,P}$ 29.3 Hz, PO_4). MALDIMS (negative mode, CHCA-matrix): m/z 617.0 $[M+H]^-$, 595.0 $[M-Na+2H]^-$, 573.0 $[M-2Na+3H]^-$, 551.0 $[M-3Na+4H]^-$.

3.8. Diallyl (benzoxazol-2-yl)-hydroxymethylphosphonate (4c)

Benzoxazole-2-carbaldehyde **2c**[15] (1.35 g, 9.18 mmol) and diallyl H-phosphonate **3** (2.23 g, 13.77 mmol) were dissolved in dry CH_2Cl_2 (15 mL) and Et_3N (0.3 mL) was added to the reaction mixture. The soln was stirred at rt for 30 min. The solvent was evaporated and flash column chromatography of the residue (20% acetone in toluene) afforded **4c** (2.55 g, 90%) as a yellow viscous liquid. $R_f = 0.30$ (40% acetone in toluene). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.63–7.57 (m, 1H, ArH), 7.43–7.36 (m, 1H, ArH), 7.24–7.14 (m, 2H, ArH), 6.05 (br s, 1H, OH), 5.87–5.67 (m, 2H, allyl CH), 5.46 (d, 1H, $J_{1',P}$ 15.6 Hz, 1′-H), 5.21–4.99 (m, 4H, allyl CH_2), 4.60–4.49 (m, 4H, allyl CH_2). ^{13}C NMR (63 MHz, $CDCl_3$): δ 161.82 (C=N), 150.71, 140.83, 132.30, 132.21, 125.26, 124.42, 120.05, 118.18, 118.15, 110.71, 68.12 (m, 1′-C), 66.92, 64.31. ^{31}P NMR (243 MHz, $CDCl_3$): δ 19.02 (s, PO_3).

3.9. Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(benzoxazol-2-yl)-diallyl phosphonatomethyl]-phosphate (6c)

Alcohol **4c** (500 mg, 1.62 mmol) and cytidine phosphoramidate **5** (1.38 g, 2.43 mmol) were co-evaporated with dry CH_2Cl_2 and dried under diminished pressure for 1 h. Then the combined reactants were dissolved in dry CH_2Cl_2 (10 mL) and tetrazole (227 mg, 3.24 mmol) was added to the reaction mixture. After stirring for 3 h at rt, *tert*-butylhydroperoxide (0.44 mL, 2.43 mmol) was added under cooling. After stirring for 2 h, Et_3N (3 mL) was added and the reaction mixture was stirred overnight. The solvent was evaporated and the residue purified by flash column chromatography (20% MeOH in EtOAc) to afford **6c** (920 mg, 67%) as a pale yellow lyophilisate. $R_f = 0.70$ (50% MeOH in EtOAc). ^{1}H NMR (250 MHz, CD_3OD): δ 8.31 (d, 0.5H, $^3J_{5,6}$ 7.6 Hz, 6-H), 8.11 (d, 0.5H, $^3J_{5,6}$ 7.6 Hz, 6-H), 7.61–7.30 (m, 5H, 5-H, Ar–H), 6.04–5.83 (m, 4H, 1″-H, 1′-H, allyl CH), 5.35–5.14 (m, 6H, 2′-H, 3′-H, allyl CH_2), 4.72–4.12 (m, 7H, $5'_{a,b}$-H, 4′-H, allyl CH_2), 3.12 (q, 6H, J 7.3 Hz, –N–CH_2–CH_3), 2.16 (s, 3H, –$OCOCH_3$), 2.02 (s, 3H, –$OCOCH_3$), 2.00 (s, 3H, $HNCOCH_3$), 1.25 (t, 9H, J 7.3 Hz, –N–CH_2–CH_3). ^{13}C NMR (63 MHz, CD_3OD): δ 172.69, 171.02, 170.78, 164.46, 164.37, 161.45, 161.26, 157.65, 157.54, 152.11, 146.29, 141.86, 133.79, 127.04, 126.01, 121.16, 118.86, 112.05, 98.80, 89.71, 89.45, 82.97 (m, 4′-C), 75.19, 71.65, 71.28, 69.82 (m, 1″-C), 65.75, 65.42, 47.98, 47.49, 24.75, 20.51, 20.37, 19.31, 9.19. ^{31}P NMR (243 MHz, CD_3OD): δ 16.33 (combination of two d, $^3J_{P,P}$ 33.9 Hz, PO_3), 0.023 (combination of two d, $^3J_{P,P}$ 33.9 Hz, PO_4). MALDIMS (positive mode, CHCA-matrix): m/z 741.4 $[M-Et_3N+H]^+$, 763.4 $[M-Et_3N+Na]^+$, 779.4 $[M-Et_3N+K]^+$.

3.10. Trisodium cytidin-5′-yl-[(benzoxazol-2-yl)-phosphonatomethyl]-phosphate (1c*h*, *l*)

A soln of **6c** (50 mg, 0.06 mmol) in dry THF (10 mL) was treated with $Pd(PPh_3)_4$ (20 mg) and dimedone (42 mg, 0.3 mmol) at rt for 12 h. The solvent was evaporated and dimedone removed from the reaction mixture by RP-18 chromatography (1:3 EtOH–water). After lyophilisation from water, the residue was dissolved in aq ammonia (4 mL) and stirred for 12 h. After lyophilisation from water, the mixture of diastereomers was separated by RP-18 HPLC (0.05 M TEAB) and finally lyophilised from water. The products were converted to their sodium salts by IR 120 (Na^+) and lyophilised to yield **1c*h*** (9 mg, 26%) and **1c*l*** (7 mg, 20%) as pale yellow solids.

3.10.1. Compound 1c*h*. HPLC: Prep. RP-18, Column A (flow 10 mL per min, 0.05 M triethylammonium bicarbonate buffer, 1% MeCN, $\chi = 260$ nm), $t_R = 28.1$ min. ^{1}H NMR (600 MHz, D_2O): δ 7.47–7.39 (m, 3H, 6-H, 5″-H, 8″-H), 7.20–7.18 (m, 2H, 6″-H, 7″-H), 5.68 (d, 1H, $^3J_{5,6}$ 7.6 Hz, 5-H), 5.44 (d, 1H, $^3J_{1',2'}$ 2.6 Hz, 1′-H), 5.20 (dd, 1H, $^2J_{1'',P}$ 14.7, $^3J_{1'',P}$ 9.0 Hz, 1″-H), 4.03–4.02 (m, 1H, $5'_a$-H), 3.90–3.87 (m, 2H, 4′-H, $5'_b$-H), 3.75–3.73 (m, 2H, 2′-H, 3′-H). ^{13}C NMR (150 MHz, D_2O): δ 165.36 (2-C), 164.13 (2″-C), 156.62 (4-C), 149.65 (4″-C), 139.84 (6-C), 139.02 (9″-C), 124.77 (6″-C), 124.13 (7″-C), 118.29 (8″-C), 110.40 (5″-C), 95.52 (5-C), 89.00 (1′-C), 81.31 ($^3J_{4',P}$ 8.7 Hz, 4′-C), 73.92 (2′-C), 70.96 ($^1J_{1'',P}$ 139.2 Hz, 1″-C), 67.54 (3′-C), 63.45 (5′-C). ^{31}P NMR (243 MHz, D_2O): δ 10.12 (d, $^3J_{P,P}$ 30.0 Hz, PO_3), 2.15 (d, $^3J_{P,P}$ 30.0 Hz, PO_4). MALDIMS (negative mode, CHCA-matrix): m/z 601.0 $[M+H]^-$, 579.0 $[M-Na+2H]^-$, 557.1 $[M-2Na+3H]^-$, 535.0 $[M-3Na+4H]^-$.

3.10.2. Compound 1c*l*. HPLC: Prep. RP-18, Column A (flow 10 mL per min, 0.05 M triethylammonium bicarbonate buffer, 1% MeCN, $\chi = 260$ nm), $t_R = 31.5$ min. ^{1}H NMR (600 MHz, D_2O): δ 7.38–7.35 (m, 2H, 5″-H, 8″-H), 7.20–7.11 (m, 3H, 6-H, 6″-H, 7″-H), 5.78 (d, 1H, $^3J_{5,6}$ 7.5 Hz, 5-H), 5.33 (d, 1H, $^3J_{1',2'}$ 2.8 Hz, 1′-H), 5.19 (dd, 1H, $^2J_{1'',P}$ 14.8, $^3J_{1'',P}$ 9.0 Hz, 1″-H), 4.15–4.13 (m, 1H, 5′$_a$-H), 3.91–3.85 (m, 2H, 4′-H, 5′$_b$-H), 3.70 (dd, 1H, $^3J_{3',4'}$ 6.6, $^3J_{3',2'}$ 5.2 Hz, 3′-H), 3.59 (dd, $^3J_{2',3'}$ 5.2, $^3J_{2',1'}$ 2.8 Hz, 2′-H). ^{13}C NMR (150 MHz, D_2O): δ 165.41 (2-C), 163.88 (2″-C), 156.57 (4-C), 149.84 (4″-C), 139.94 (6-C), 138.99 (9″-C), 124.50 (6″-C), 123.88 (7″-C), 118.21 (8″-C), 110.66 (5″-C), 95.73 (5-C), 88.60 (1′-C), 80.90 ($^3J_{4',P}$ 9.8 Hz, 4′-C), 73.76 (2′-C), 70.94 ($^1J_{1'',P}$ 129.5 Hz, 1″-C), 67.15 (3′-C), 62.54 (5′-C). ^{31}P NMR (243 MHz, D_2O): δ 10.11 (d, $^3J_{P,P}$ 30.2 Hz, PO_3), 2.65 (d, $^3J_{P,P}$ 30.2 Hz, PO_4). MALDIMS (negative mode, CHCA-matrix): *m*/*z* 601.2 $[M+H]^-$, 579.2 $[M-Na+2H]^-$, 557.2 $[M-2Na+3H]^-$, 535.2 $[M-3Na+4H]^-$.

3.11. Diallyl (benzothiophen-2-yl)-hydroxymethylphosphonate (4d)

Benzothiophene-2-carbaldehyde **2d**[14] (1.9 g, 11.73 mmol) and diallyl H-phosphonate **3** (2.85 g, 17.59 mmol) were dissolved in dry CH_2Cl_2 (20 mL) and Et_3N (0.3 mL) was added to the reaction mixture. The soln was stirred at rt for 24 h. The solvent was evaporated and flash column chromatography of the residue (20% acetone in toluene) afforded **4d** (3.62 g, 95%) as a colourless solid. Mp 51 °C. $R_f = 0.36$ (40% acetone in toluene). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (d, 1H, *J* 7.5 Hz, ArH), 7.67 (d, 1H, *J* 7.4 Hz, ArH), 7.35 (d, 1H, *J* 2.9 Hz, ArH), 7.31–7.27 (m, 2H, ArH, 3-H), 5.89–5.84 (m, 2H, allyl CH), 5.37–5.13 (m, 5H, allyl CH_2, 1′-H), 4.57–4.53 (m, 4H, allyl CH_2). ^{13}C NMR (63 MHz, $CDCl_3$): δ 141.13, 139.85, 133.13, 133.04, 132.64, 132.54, 124.62, 124.03, 123.04, 122.90, 122.63, 118.97, 118.58, 69.32 (m, 1′-C), 66.66, 66.61. ^{31}P NMR (243 MHz, $CDCl_3$): δ 21.84 (s, PO_3). MALDIMS (positive mode, DHB-matrix): *m*/*z* 347.3 $[M+Na]^+$, 363.3 $[M+K]^+$.

3.12. Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(benzothiophen-2-yl)-diallyl phosphonatomethyl]-phosphate (6d)

Alcohol **4d** (500 mg, 1.54 mmol) and cytidine phosphoramidate **5** (1.3 g, 2.32 mmol) were co-evaporated with dry CH_2Cl_2 and dried under diminished pressure for 1 h. Then the combined reactants were dissolved in dry CH_2Cl_2 (10 mL) and tetrazole (216 mg, 3.08 mmol) was added to the reaction mixture. After stirring for 3 h at rt, *tert*-butylhydroperoxide (0.42 mL, 2.32 mmol) was added under cooling. After stirring for 2 h, Et_3N (3 mL) was added and the reaction mixture stirred overnight. The solvent was evaporated and the residue purified by flash column chromatography (20% MeOH in EtOAc) to afford **6d** (850 mg, 64%) as a pale yellow coloured lyophilisate. $R_f = 0.70$ (50% MeOH in EtOAc). ^{1}H NMR (250 MHz, CD_3OD): δ 8.30 (d, 0.5H, $^3J_{5,6}$ 7.5 Hz, 6-H), 8.18 (d, 0.5H, $^3J_{5,6}$ 7.5 Hz, 6-H), 7.84–7.70 (m, 2H, Ar–H), 7.52–7.28 (m, 4H, 5-H, Ar–H), 6.10–5.86 (m, 4H, 1″-H, 1′-H, allyl CH), 5.38–5.15 (m, 6H, 2′-H, 3′-H, allyl CH_2), 4.70–3.97 (m, 7H, 5′$_{a,b}$-H, 4′-H, allyl CH_2), 3.09 (q, 6H, *J* 7.3 Hz, –N–CH_2–CH_3), 2.19 (s, 3H, –OCOCH_3), 2.18 (2s, 3H, –OCOCH_3), 2.03 (2s, 3H, HNCOCH_3), 1.27 (t, 9H, *J* 7.3 Hz, –N–CH_2–CH_3). ^{13}C NMR (63 MHz, CD_3OD): δ 172.82, 171.13, 171.07, 170.91, 164.41, 157.81, 141.48, 140.38, 134.16, 134.07, 125.95, 125.63, 124.98, 118.75, 98.80, 88.21, 83.17, 75.34, 75.21, 72.17, 69.57, 69.46 (m, 1″-C), 65.71, 65.60, 47.49, 24.85, 20.54, 20.49, 9.24. ^{31}P NMR (243 MHz, CD_3OD): δ 19.37 (combination of two d, $^3J_{P,P}$ 31.9 Hz, PO_3), 0.017 (combination of two d, $^3J_{P,P}$ 31.9 Hz, PO_4). MALDIMS (positive mode, CHCA-matrix): *m*/*z* 756.4 $[M-Et_3N+H]^+$, 778.5 $[M-Et_3N+Na]^+$, 794.5 $[M-Et_3N+K]^+$.

3.13. Trisodium cytidin-5′-yl-[(benzothiophen-2-yl)-phosphonatomethyl]-phosphate (1d)

A soln of **6d** (200 mg, 0.23 mmol) in dry THF (20 mL) was treated with $Pd(PPh_3)_4$ (50 mg) and dimedone (164 mg, 1.17 mmol) at rt for 12 h. The solvent was evaporated and dimedone removed from the reaction mixture by RP-18 chromatography (ethanol/water, 1:3). After lyophilisation from water, the residue was dissolved in aq ammonia (4 mL) and stirred for 12 h. After lyophilisation from water, the product was purified (not separated) by RP-18 HPLC (0.05 M TEAB) and finally lyophilised from water. The product was converted to its sodium salt by IR 120 (Na^+) and was lyophilised to yield **1d** (70 mg, 49%) as a pale yellow solid. HPLC: Prep. RP-18, Column A (flow 10 mL per min, 0.05 M triethylammonium bicarbonate buffer, 3% MeCN, $\chi = 260$ nm), $t_R = 24.8$ min. ^{1}H NMR (600 MHz, D_2O): δ 7.72 (d, 0.5H, $^3J_{8'',7''}$ 6.5 Hz, 8″-H), 7.63 (d, 0.5H, $^3J_{8'',7''}$ 6.5 Hz, 8″-H), 7.50 (d, 0.5H, $^3J_{5'',6''}$ 6.7 Hz, 5″-H), 7.47 (d, 0.5H, $^3J_{5'',6''}$ 6.7 Hz, 5″-H), 7.41 (d, 0.5H, $^3J_{6,5}$ 7.6 Hz, 6-H), 7.23–7.11 (m, 3.5H, 6-H, 6″-H, 7″-H, 3″-H), 5.77 (d, 0.5H, $^3J_{5,6}$ 7.6 Hz, 5-H), 5.75 (d, 0.5H, $^3J_{5,6}$ 7.6 Hz, 5-H), 5.43 (d, 0.5H, $^3J_{1',2'}$ 3.2 Hz, 1′-H), 5.36–5.30 (m, 1.5H, 1′-H, 1″-H), 3.98 (bd, 0.5H, *J* 12.0 Hz, 5′$_a$-H), 3.92 (bd, 0.5H, *J* 12.0 Hz, 5′$_a$-H), 3.83–3.73 (m, 2H, 5′$_b$-H, 4′-H), 3.45–3.24 (m, 2H, 2′-H, 3′-H). ^{13}C NMR (150 MHz, D_2O): δ 165.13 (2-C), 156.33 (4-C), 156.17 (4-C), 141.48 (4″-C), 141.42 (4″-C), 140.25 (6-C), 140.15 (6-C), 139.23 (2″-C), 139.15 (2″-C), 138.38 (9″-C), 138.33 (9″-C), 123.81 (6″-C), 123.77 (6″-C), 123.65 (7″-C),

123.60 (7″-C), 123.53 (3″-C), 123.48 (3″-C), 122.80 (8″-C), 122.69 (8″-C), 121.74 (5″-C), 121.52 (5″-C), 95.55 (5-C), 89.12 (1′-C), 89.06 (1′-C), 81.65 ($^3J_{4',P}$ 9.3 Hz, 4′-C), 81.12 ($^3J_{4',P}$ 10.2 Hz, 4′-C), 73.72 (2′-C), 73.66 (2′-C), 73.34 ($^1J_{1'',P}$ 156.1, $^2J_{1'',P}$ 9.3 Hz, 1″-C), 73.11 ($^1J_{1'',P}$ 156.0, $^2J_{1'',P}$ 9.0 Hz, 1″-C), 67.80 (3′-C), 67.09 (3′-C), 63.39 (5′-C), 62.29 (5′-C). ^{31}P NMR (243 MHz, D_2O): δ 13.81 (d, $^3J_{P,P}$ 33.0 Hz, PO_3), 13.62 (d, $^3J_{P,P}$ 33.4 Hz, PO_3), 2.94 (d, $^3J_{P,P}$ 33.4 Hz, PO_4), 2.48 (d, $^3J_{P,P}$ 33.0 Hz, PO_4). MALDIMS (negative mode, CHCA-matrix): *m/z* 616.3 $[M+H]^-$, 572.3 $[M-2Na+3H]^-$, 550.3 $[M-3Na+4H]^-$.

3.14. Diallyl hydroxy-(thiophen-2-yl)-methylphosphonate (4e)

Thiophene-2-carbaldehyde **2e** (2 g, 17.83 mmol) and diallyl H-phosphonate **3** (4.33 g, 26.74 mmol) were dissolved in dry CH_2Cl_2 (20 mL) and Et_3N (0.3 mL) was added to the reaction mixture. The soln was stirred at rt for 24 h. The solvent was evaporated and flash column chromatography of the residue (20% acetone in toluene) afforded **4e** (4.6 g, 94%) as a yellow viscous liquid. $R_f = 0.30$ (40% acetone in toluene). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25 (d, 1H, $^3J_{5,4}$ 4.4 Hz, 5-H), 7.15–7.11 (m, 1H, 3-H), 6.95 (t, 1H, *J* 4.0 Hz, 4-H), 5.86–5.82 (m, 2H, allyl CH), 5.29–5.14 (m, 5H, allyl CH_2, 1′-H), 4.51–4.44 (m, 4H, allyl CH_2). ^{13}C NMR (63 MHz, $CDCl_3$): δ 139.90, 133.19, 133.09, 127.22, 127.19, 126.70, 126.58, 126.17, 126.12, 118.51, 118.45, 68.74 (m, 1′-C), 67.90, 66.07. ^{31}P NMR (243 MHz, $CDCl_3$): δ 22.19 (s, PO_3). MALDIMS (positive mode, DHB-matrix): *m/z* 297.2 $[M+Na]^+$, 313.1 $[M+K]^+$.

3.15. Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl)-[(diallylphosphonato)-(thiophen-2-yl)-methyl]-phosphate (6e)

Alcohol **4e** (500 mg, 1.82 mmol) and cytidine phosphoramidate **5** (1.55 g, 2.73 mmol) were co-evaporated with dry CH_2Cl_2 and dried under diminished pressure for 1 h. Then the combined reactants were dissolved in dry CH_2Cl_2 (10 mL) and tetrazole (255 mg, 3.64 mmol) was added to the reaction mixture. After stirring for 3 h at rt, *tert*-butylhydroperoxide (0.5 mL, 2.73 mmol) was added under cooling. After stirring for 2 h, Et_3N (3 mL) was added and the reaction mixture stirred overnight. The solvent was evaporated and the residue purified by flash column chromatography (20% MeOH in EtOAc+1% Et_3N) to afford **6e** (1.2 g, 82%) as a pale yellow lyophilisate. $R_f = 0.78$ (50% MeOH in EtOAc). ^{1}H NMR (250 MHz, CD_3OD): δ 8.36 (d, 0.5H, $^3J_{5,6}$ 7.5 Hz, 6-H), 8.27 (d, 0.5H, $^3J_{5,6}$ 7.5 Hz, 6-H), 7.51–7.43 (m, 2H, 5-H, 5″-H), 7.27 (d, 1H, $^3J_{3'',4''}$ 2.6 Hz, 3″-H), 7.00 (dd, 1H, $^3J_{4'',5''}$ 3.8, $^3J_{4'',3''}$ 2.6 Hz, 4″-H), 6.11 (dd, 1H, $^2J_{1''P}$ 11.4, $^3J_{1''P}$ 5.5 Hz, 1″-H), 5.91–5.81 (m, 3H, 1′-H, allyl CH), 5.39–5.16 (m, 6H, 2′-H, 3′-H, allyl CH_2), 4.67–3.97 (m, 7H, $5'_{a,b}$-H, 4′-H, allyl CH_2), 3.16 (q, 6H, *J* 7.3 Hz, –N–CH_2–CH_3), 2.18 (s, 3H, –$OCOCH_3$), 2.09 (s, 3H, –$OCOCH_3$), 2.05/2.04 (2s, 3H, $HNCOCH_3$), 1.31 (t, 9H, *J* 7.3 Hz, –N–CH_2–CH_3). ^{13}C NMR (63 MHz, CD_3OD): δ 172.84, 171.16, 170.93, 164.50, 157.38, 146.82, 138.37, 134.21, 134.12, 129.13, 128.24, 127.81, 118.54, 98.91, 89.41, 89.28, 83.17, 75.37, 72.39, 71.93, 69.42 (m, 1″-C), 65.16, 47.55, 24.65, 20.55, 20.34, 19.37, 9.19. ^{31}P NMR (243 MHz, CD_3OD): δ 19.78 (combination of two d, $^3J_{P,P}$ 33.9 Hz, PO_3), 0.166 (combination of two d, $^3J_{P,P}$ 33.9 Hz, PO_4). MALDIMS (positive mode, CHCA-matrix): *m/z* 728.5 $[M-Et_3N+Na]^+$, 744.4 $[M-Et_3N+K]^+$.

3.16. tris-Triethylammonium cytidin-5′-yl-[phosphonato-(thiophen-2-yl)-methyl]-phosphate (1e*h*, *l*)

A soln of **6e** (125 mg, 0.155 mmol) in dry THF (10 mL) was treated with $Pd(PPh_3)_4$ (30 mg) and dimedone (108 mg, 0.78 mmol) at rt for 12 h. The solvent was evaporated and dimedone removed from the reaction mixture by RP-18 chromatography (ethanol/water, 1:3). After lyophilisation from water, the residue was dissolved in aq ammonia (4 mL) and stirred for 12 h. After lyophilisation from water, the mixture of diastereomers was separated by RP-18 HPLC (0.05 M TEAB) and finally lyophilised from water to give **1e*h*** (18 mg, 15%) and **1e*l*** (15 mg, 12%) as pale yellow solids.

3.16.1. Compound 1e*h*. HPLC: Prep. RP-18, Column A (flow 10 mL per min, 0.05 M triethylammonium bicarbonate buffer, 2% MeCN, $\chi = 260$ nm), $t_R = 12.5$ min. ^{1}H NMR (600 MHz, D_2O): δ 7.68 (d, 1H, $^3J_{6,5}$ 7.6 Hz, 6-H), 7.27 (d, 1H, $^3J_{5'',4''}$ 5.0 Hz, 5″-H), 7.04 (d, 1H, $^3J_{3'',4''}$ 3.7 Hz, 3″-H), 6.87 (dd, 1H, $^3J_{4'',5''}$ 5.0, $^3J_{4'',3''}$ 3.7 Hz, 4″-H), 5.97 (d, 1H, $^3J_{5,6}$ 7.6 Hz, 5-H), 5.77 (d, 1H, $^3J_{1',2'}$ 5.1 Hz, 1′-H), 5.30 (dd, 1H, $^2J_{1'',P}$ 13.7, $^3J_{1'',P}$ 9.9 Hz, 1″-H), 3.94–3.91 (m, 2H, 2′-H, 4′-H), 3.84–3.51 (m, 3H, 3′-H, $5'_{a,b}$-H), 3.05 (q, 18H, *J* 7.3 Hz, NCH_2CH_3), 1.14 (t, 27H, *J* 7.3 Hz, NCH_2CH_3). ^{13}C NMR (150 MHz, D_2O): δ 165.24 (2-C), 156.83 (4-C), 140.84 (6-C), 138.72 (2″-C), 127.22 (3″-C), 126.12 (5″-C), 126.00 (4″-C), 96.06 (5-C), 88.15 (1′-C), 82.50 ($^3J_{4',P}$ 9.0 Hz, 4′-C), 73.62 (2′-C), 71.49 ($^1J_{1'',P}$ 164.1 Hz, 1″-C), 69.04 (3′-C), 63.94 (5′-C), 46.06 (NCH_2CH_3), 7.64 (NCH_2CH_3). ^{31}P NMR (243 MHz, D_2O): δ 14.75 (d, $^3J_{P,P}$ 33.6 Hz, PO_3), 1.90 (d, $^3J_{P,P}$ 33.6 Hz, PO_4). MALDIMS (positive mode, CHCA-matrix): *m/z* 522.5 $[M-3Et_3N+Na]^+$, 568.6 $[M-Et_3N+3Na]^+$.

3.16.2. Compound 1e*l*. HPLC: Prep. RP-18, Column A (flow 10 mL per min, 0.05 M triethylammonium bicarbonate buffer, 2% MeCN, $\chi = 260$ nm), $t_R = 16.8$ min. ^{1}H NMR (600 MHz, D_2O): δ 7.59 (d, 1H, $^3J_{6,5}$ 7.6 Hz,

6-H), 7.21 (d, 1H, $^{3}J_{5'',4''}$ 5.0 Hz, 5″-H), 7.01 (d, 1H, $^{3}J_{3'',4''}$ 3.6 Hz, 3″-H), 6.81 (dd, 1H, $^{3}J_{4'',5''}$ 5.0, $^{3}J_{4'',3''}$ 3.6 Hz, 4″-H), 5.96 (d, 1H, $^{3}J_{5,6}$ 7.6 Hz, 5-H), 5.71 (d, 1H, $^{3}J_{1',2'}$ 4.3 Hz, 1′-H), 5.28 (dd, 1H, $^{2}J_{1'',P}$ 13.6, $^{3}J_{1'',P}$ 10.0 Hz, 1″-H), 3.91–3.89 (m, 2H, 2′-H, 4′-H), 3.82–3.74 (m, 3H, 3′-H, $5'_{a,b}$-H), 3.05 (q, 18H, *J* 7.3 Hz, NCH_2CH_3), 1.14 (t, 27H, *J* 7.3 Hz, NCH_2CH_3). ^{13}C NMR (150 MHz, D_2O): δ 165.50 (2-C), 157.01 (4-C), 140.83 (6-C), 139.00 (2″-C), 127.01 (3″-C), 125.95 (4″-C), 125.68 (5″-C), 95.98 (5-C), 88.57 (1′-C), 82.16 ($^{3}J_{4',P}$ 9.3 Hz, 4′-C), 73.66 (2′-C), 71.71 ($^{1}J_{1'',P}$ 155.3 Hz, 1″-C), 68.42 (3′-C), 63.24 (5′-C), 46.06 (NCH_2CH_3), 7.64 (NCH_2CH_3). ^{31}P NMR (243 MHz, D_2O): δ 14.53 (d, $^{3}J_{P,P}$ 33.0 Hz, PO_3), 2.42 (d, $^{3}J_{P,P}$ 33.0 Hz, PO_4). MALDIMS (positive mode, CHCA-matrix): *m/z* 522.6 $[M-3Et_3N+Na]^+$, 568.7 $[M-Et_3N+3Na]^+$.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. B.M. is grateful for an Alexander von Humboldt Fellowship.

References

1. (a) Morgenthaler, J.; Kemner, J.; Brossmer, R. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 860–866; (b) Rosenberg, A. *Biology of Sialic Acids*; Plenum: New York, 1995; (c) Reutter, W.; Stäsche, R.; Stehling, P.; Baum, O. In *Glycosciences*; Gabius, H. J., Gabius, S., Eds.; Chapman and Hall: Weinheim, 1997; pp 245–259; (d) Schauer, R. *Glycoconjugate J.* **2000**, *17*, 485–499; (e) Dall'Olio, F.; Chiricolo, M. *Glycoconjugate J.* **2001**, *18*, 841–850.
2. (a) Jogeeswaran, G. *Adv. Cancer Res.* **1983**, *38*, 289–350; (b) Collard, J. G.; Schijen, J. F.; Bikker, A.; LaRiviere, G.; Bolscher, J. G. M.; Roos, E. *Cancer Res.* **1986**, *46*, 3521–3527; (c) Petrick, A. T.; Meterissian, S.; Steele, G.; Thomas, P. *Clin. Exp. Metastasis* **1994**, *12*, 108–116; (d) Hennet, T.; Chui, D.; Paulson, J. C.; Marth, J. D. *Proc. Natl. Acad. Sci.* **1998**, *95*, 4504–4509.
3. Harvey, B. E.; Toth, C. A.; Wagner, H. E.; Steele, G. D.; Thomas, P. *Cancer Res.* **1992**, *52*, 1775–1779.
4. (a) Schaub, C.; Müller, B.; Schmidt, R. R. *Glycoconjugate J.* **1998**, *15*, 345–354; (b) Müller, B.; Martin, T. J.; Schaub, C.; Schmidt, R. R. *Tetrahedron Lett.* **1988**, *39*, 509–512.
5. (a) Brunner, M.; Horenstein, B. A. *Biochemistry* **2000**, *39*, 2261–2268; (b) Horenstein, B. A.; Brunner, M. *Biochemistry* **1998**, *37*, 289–297; (c) Horenstein, B. A.; Brunner, M. *J. Am. Chem. Soc.* **1996**, *118*, 10371–10379.
6. Müller, B.; Schaub, C.; Schmidt, R. R. *Angew. Chem.* **1998**, *110*, 3021–3024; . *Angew. Chem., Int. Ed.* **1998**, *37*, 2893–2897.
7. (a) Amann, F.; Schaub, C.; Müller, B.; Schmidt, R. R. *Chem. Eur. J.* **1998**, *4*, 1106–1115; (b) Schaub, C.; Müller, B.; Schmidt, R. R. *Eur. J. Org. Chem.* **2000**, 1745–1758; (c) Dufner, G.; Schwörer, R.; Müller, B.; Schmidt, R. R. *Eur. J. Org. Chem.* **2000**, 1467–1482.
8. Schwörer, R.; Schmidt, R. R. *J. Am. Chem. Soc.* **2002**, *124*, 1632–1637.
9. (a) Skropeta, D.; Schmidt, R. R. *Tetrahedron: Asymmetry* **2003**, *14*, 265–273; (b) Skropeta, D.; Schwörer, R.; Schmidt, R. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3351–3354; (c) Skropeta, D.; Schwörer, R.; Haag, T.; Schmidt, R. R. *Glycoconjugate J.* **2004**, *21*, 205–219, 221–225.
10. (a) Jung, K.-H.; Schmidt, R. R. In *Carbohydrate-Based Drug Discovery*; Wong, C. H., Ed.; Wiley-VCH, 2003; pp 609–659; (b) Jung, K.-H.; Schwörer, R.; Schmidt, R. R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 275–289.
11. (a) Gibbs, D. E.; Larsen, C. *Synthesis* **1984**, 410–412; (b) Felber, R.; Lohse, P. A. *Tetrahedron Lett.* **1998**, *39*, 2067–2070.
12. Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859–1862.
13. (a) Schmidt, R. R.; Frische, K. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1747–1750; (b) Kajihara, Y.; Ebata, T.; Koseki, K.; Kodama, H.; Matsushita, H.; Hashimoto, H. *J. Org. Chem.* **1995**, *60*, 5732–5735.
14. Rohloff, J. C.; Alfredson, T. V.; Schwartz, M. A. *Tetrahedron Lett.* **1994**, *35*, 1011–1014.
15. Vetelino, M. G.; Coe, J. W. *Tetrahedron Lett.* **1994**, *35*, 219–222.