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Asymmetric synthesis and affinity of potent sialyltransferase inhibitors based on transition-state analogues

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Inhibitors that are structurally related to the transition-state model of the proposed S_N1-type mechanism of sialyl transfer, exhibit particularly high binding affinities to α(2–6)sialyltransferases. Furthermore, replacing the neuraminyl residue with a simple aryl or hetaryl ring and substituting the carboxylate group for a phosphonate moiety, improves both binding affinity and synthetic accessibility. Herein we report on the synthesis and inhibition of a wide range of novel, potent transition-state analogue based α(2-6)sialyltransferase inhibitors comprising a planar anomeric carbon, an increased distance between the anomeric carbon and the CMP leaving group, and at least two negative charges. We also present a short, efficient asymmetric synthesis of the most promising benzyl inhibitors, providing rapid access to large quantities of highly potent, stereochemically-pure (>96% *de*) inhibitors for further biological investigation (*e.g.* (*R*)-3b, $K_i = 70$ nM).
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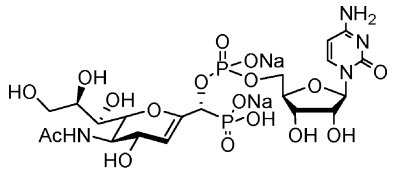
Introduction

Sialyltransferases catalyse the addition of sialic acid to glycoconjugate acceptors, producing α-sialosides that influence many important biological processes, including cell adhesion and inflammation [1–7]. Recently, a correlation has also been discovered between α(2-6)-sialylation of *N*-acetyllactosamine and B lymphocyte activation and immune function [8]. Sialyltransferase inhibitors are thus valuable tools in elucidating the role of sialyl residues in biological systems [9].

Independent of both their source and broad acceptor specificity, the various sialyltransferases employ cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) as the sialyl donor. Transition-state analogues of CMP-Neu5Ac (*e.g.* **1**) based on the proposed mechanism of sialyl transfer involving partial dissociation of the CMP moiety and the formation of a planar oxocarbenium structure in the transition-state [10,11,16–18], show high affinity to sialyltransferases (Scheme 1) [10–15]. In a previous study we have shown that the neuraminyl residue of transition-state analogues of CMP-Neu5Ac can be replaced by aryl rings, to produce readily accessible, potent aromatic inhibitors of α(2-6)sialyltransferase from rat liver [13].



(*R*)-(**1**)

Herein we report on the synthesis and inhibition of new examples of α(2-6)sialyltransferase inhibitors based on transition-state analogues. Further examples of benzyl and phenylethyl based sialyltransferase inhibitors bearing either single or multiple carboxylate or phosphonate substituents are presented, in order to assess both the effect of: (i) an increased distance between the planar anomeric carbon and the CMP leaving group, and (ii) the number of negative charges close to the glycosylation cleavage site (Figure 1). A wide variety of novel *m*- and *p*-substituted benzyl inhibitors (**3a**–**f**) are also described, in

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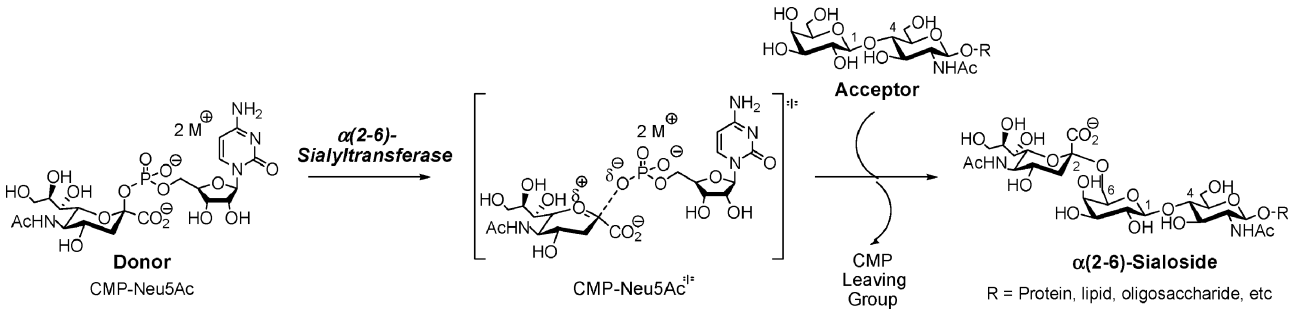


Scheme 1. Mechanism of sialyl transfer.

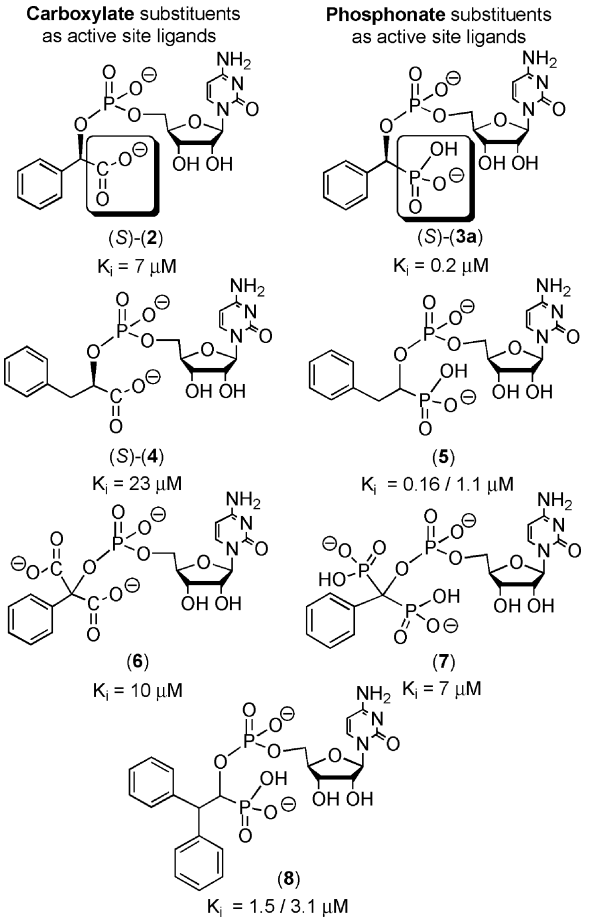


Figure 1. Comparison of inhibition of α(2-6)sialyltransferase by benzyl and phenylethyl transition-state analogues containing carboxylate and phosphonate substituents.

order to investigate the influence on inhibition of electron-donating and electron-withdrawing substituents on the aromatic ring. Furthermore, a diverse range of aryl and hetaryl inhibitors such as pyridyl (**3g–i**), naphthyl (**3j**,**k**), quinolinyl (**3l**) and pyrimidonyl (**3m**) inhibitors are also presented (Scheme 4).

The aryl phosphonate inhibitors reported here show dramatically improved affinities to α(2-6)sialyltransferase compared to the earlier series of carboxylato compounds [13]. The attractive levels of enzyme affinity observed have led us to develop an asymmetric route, which allows assignment of the configuration of the newly generated stereogenic centre, as well as providing rapid access to large scale, stereochemically pure sialyltransferase inhibitors for further biological investigation. Thus, herein we also present a short, efficient stereoselective synthesis of the most promising benzyl inhibitors (*S*)-**3a–c** and (*R*)-**3a–c** in high diastereomeric excess (>96–98% *de*).

Results and discussion

Synthesis

Analogues such as (*R*)-**1**, derived from the transition-state of the proposed reaction course (CMP-Neu5Ac$^{\neq}$ in Scheme 1), have been shown by us to exhibit high enzyme affinity to rat liver α(2-6)sialyltransferase [12,13]. An earlier investigation into the importance of the sialyl moiety for binding affinity, which involved a range of CMP-quinic acid donor analogues, revealed that the complete Neu5Ac residue may not be required for high enzyme affinity [10,12]. Thus, we reasoned that it should be possible to replace the neuraminyl residue with aryl and hetaryl rings to produce readily accessible potent aromatic inhibitors of α(2-6)sialyltransferase [13].

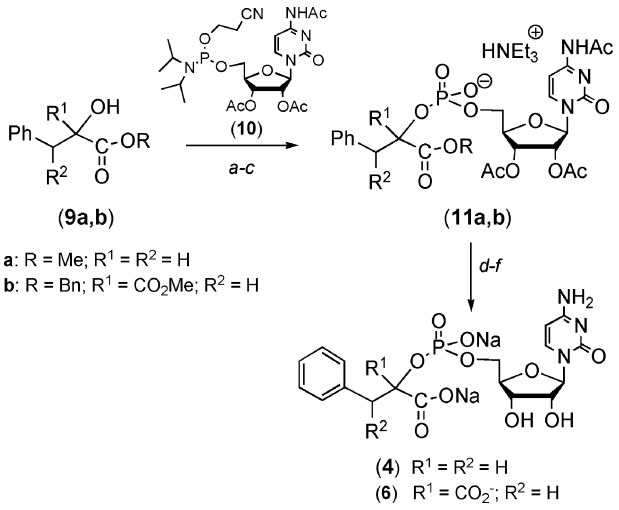
Previous studies comparing the sialyltransferase inhibition activity of the simple benzylcarboxylato (**2**) and benzylphosphonato (**3a**) transition-state analogue inhibitors had shown that replacement of the carboxylate group by a phosphonate group increases the binding affinity by approximately one order of magnitude (Figure 1) [13]. To further examine this effect we have compared the inhibition values of the phenylethylcarboxylato (**4**) [13]/phenylethylphosphonato (**5**) pair, along with the novel derivatives **6** and **7**. The latter pair, which contain an additional carboxylate and phosphonate group at the α-centre (**6** and **7**, resp.), also reveal the role of negative charges on binding to sialyltransferase (Figure 1).

The novel di-carboxylato inhibitor **6** was prepared from dibenzyl benzyltartronate (**9b**), according to the reported procedure for the preparation of the mono-carboxylato inhibitor **4** from methyl (*S*)- and (*R*)-phenyllactate (**9a**) [13]. The method involves condensation with cytidine phosphitamide (**10**) in the

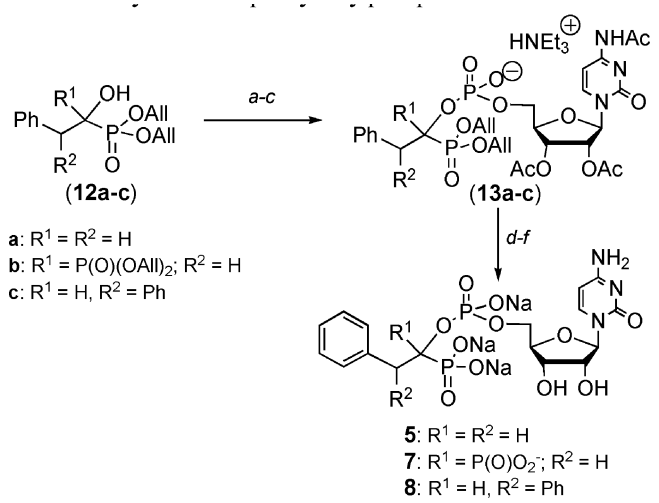
presence of tetrazole, followed by oxidation with *tert*-butyl hydroperoxide, and base-catalysed cleavage of the cyanoethyl group, which furnishes the fully protected forms of the inhibitors, **11a** and **11b** respectively. Subsequent removal of the carboxylate protecting groups by either saponification (for **4**) or catalytic hydrogenation (for **6**), followed by reverse-phase (RP) C-18 column chromatography (1:3 EtOH-H_2O), *O*/*N*-deacetylation with 25% NH_3 (aq.), repeated RP column chromatography (95:5 H_2O-CH_3CN), and conversion to the sodium salt form by ion-exchange (IR 120 Na^+), gave the desired inhibitors (*S*)- and (*R*)-**4** and a diastereomeric mixture of **6** (Scheme 2).

The analogous mono- and di-phosphonato inhibitors (**5**) and (**7**), along with diphenyl phosphonato inhibitor (**8**) were prepared in a similar manner by condensing cytidine phosphitamide (**10**) with the corresponding α-hydroxyphosphonates **12a–c**. Pd-mediated deprotection of the di- and tetra-allyl protected intermediates **13a–c**, followed by the *O*/*N*-deacetylation and purification steps described above gave the target inhibitors **5**, **7** and **8** (Scheme 3).

In order to assess the role of electron-donating and electron-withdrawing substituents on enzyme inhibition, a variety of novel *m*- and *p*-substituted benzyl inhibitors (**3a–f**), were also prepared by coupling the corresponding α-hydroxybenzylphosphonates with cytidine phosphitamide, as described above. Furthermore, in order to examine the influence of aryl and hetaryl rings on enzyme inhibition, a diverse range of inhibitors including pyridyl (**3g–i**), naphthyl (**3j**,**k**), quinolinyl (**3l**) and pyrimidonyl (**3m**) inhibitors were also prepared from their corresponding α-hydroxyphosphonates in a similar manner (Scheme 4).



Scheme 2. Synthesis of phenylethylcarboxylato inhibitors. *Reagents and Conditions:* (a) 1.5 eq. (**10**), 2 eq. tetrazole, CH_2Cl_2, RT, 3 h; (b) 1.5 eq. *t*-BuOOH, RT, 1 h; (c) 50 eq. NEt_3, RT, 18 h; (d) For **9a**: NaOH, MeOH/H_2O, RT, 18 h; For **9b**: 10% Pd / C, H_2(g), MeOH, NEt_3, RT, 10–40 min; (e) 25% NH_3-H_2O, RT, 18 h; (f) IR 120 Na^+.



Scheme 3. Synthesis of phenylethylphosphonato inhibitors. *Reagents and Conditions:* (a) 1.5 eq. (**10**), 2 eq. tetrazole, CH_2Cl_2, RT, 3 h; (b) 1.5 eq. *t*-BuOOH, RT, 1 h; (c) 50 eq. NEt_3, RT, 18 h; (d) 20 mol. % $Pd(Ph_3)_4$, 10.0 eq. dimedone, THF, RT, 18 h; (e) 25% NH_3-H_2O, RT, 18 h; (f) IR 120 Na^+.

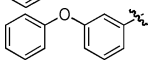
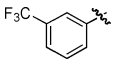
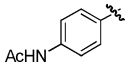
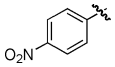
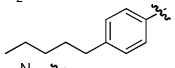
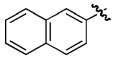
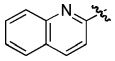
The phenylethyl phosphonato inhibitors **5**, **7**, and **8** and the aryl phosphonato inhibitors **3a–m** were obtained as diastereomeric mixtures. The diastereomerically pure inhibitors required for the enzyme inhibition assays, were obtained by RP-HPLC separation and referred to as (*h*)- and (*l*)-derivatives based on their different R_f values (*h*igh, *l*ow), in those cases where the configuration at the nascent stereogenic centre could not readily be assigned.

Biological activity

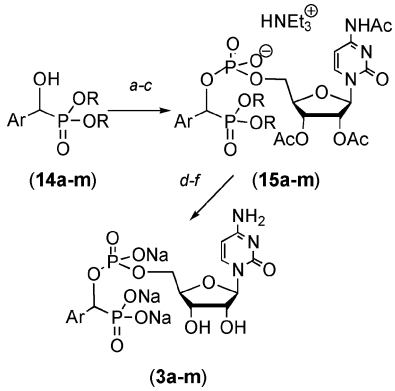
Measurement of the inhibition constants K_i of the various transition-state analogues against α(2-6)-sialyltransferase from rat liver is based on a previously reported assay [10]. It was found that replacement of the carboxylate group of **4** by a phosphonate group in the analogous phenylethyl inhibitor **5**, increases the binding affinity by approximately one order of magnitude, as has been shown previously [13] for the benzyl inhibitors **2** and **3** (Table 1). Thus, it appears that two negative charges at the glycosylation cleavage site provide more efficient binding to the active site than only one.

The configuration at the carbon atom bearing the CMP moiety results in approximately 4-fold higher K_i values in the case of phosphoryl derivatives (Entries 3 *vs* 4 and 7 *vs* 8, Table 1), but has a much smaller influence on the binding affinity of the carboxylate containing inhibitors (*R*)-/(*S*)-**2** and (*R*)-/(*S*)-**4**. Interestingly, the simple benzyl and phenylethyl phosphonato derivatives, in particular (*S*)-**3a** and (*h*)-**5** (where *h* refers to *h*igh R_f value) exhibit even higher binding affinities to α(2-6)sialyltransferase than the more structurally complex neuraminyl derivative (*R*)-**1** ($K_i = 0.35 \pm 0.01$ μM, [13]).

The presence of additional phosphonate and carboxylate groups in derivatives **6** and **7**, led in both cases to a decrease in

Cpd	*Ar*	*R*	*Overall yield*[a] *(%)*
a		All	31
b		All	31
c		Bn	33
d		Bn	31
e		All	28
f		All	42
g		All	34
h		All	46
i		All	59[b]
j		All	36
k		All	22
l		All	19
m		All	20

[a]Over 3 steps, from commercially available aldehyde.
[b]Over 2 steps.



Scheme 4. Synthesis of aryl sialyltransferase inhibitors. *Reagents and Conditions:* (a) 1.5 eq. (**10**), 2.0 eq. tetrazole, CH_2Cl_2, RT, 3 h; (b) 1.5 eq. *t*-BuOOH, RT, 1 h; (c) NEt_3, RT, 18 h; (d) For All: 20 mol. % $Pd(Ph_3)_4$, 10.0 eq. dimedone, THF, RT, 18 h, For Bn: 10% Pd/C, H_2(g), MeOH, NEt_3, RT, 10–40 min; (e) 25% NH_3-H_2O, RT, 18 h; (f) IR 120 Na^+.

inhibitory activity. These results suggest that the optimum motif for sialyltransferase binding comprises a phosphate linked CMP motif and an α-phosphonate moiety, where the two negative oxyanion charges (assuming only mono-deprotonation of the phosphonic acid moiety occurs at physiological pH) are separated by five bonds.

All of the novel *m*- and *p*-substituted benzyl inhibitors (**3a–f**) prepared showed competitive inhibition against rat liver α(2-6)sialyltransferase, with sub-micro molar K_i values, irrelevant of whether the substituent was electron-donating or electron-withdrawing (*c.f.* **3d/3e**, Entries 7/8, Table 2). These results present a further example of the possibility of replacing the neuraminic acid moiety with simple aryl systems, to produce readily accessible, highly active sialyltransferase inhibitors. Furthermore, the most potent inhibitor in the benzyl phosphonato series is the (*R*)-diastereomer of the *m*-phenoxy derivative (*R*)-**3b**, with the m-phenoxy group as mimic of the Neu5Ac side chain; the K_i value is in the nanomolar range (70 nM), three orders of magnitude greater affinity than the natural substrate CMP-Neu5Ac.

Inhibitors based on hetaryl ring systems such as the 2-pyridyl (**3g**) and 4-pyridyl derivatives (**3h**) also proved to be excellent inhibitors of α(2-6)sialyltransferase (Scheme 4). Apart from the *N*-methyl pyridinium derivative **3i** which showed a loss of activity, the inhibition values of the pyridyl inhibitors were comparable to those of the benzyl inhibitors, with the best pyridyl inhibitor, (*h*)-**3g**, also showing a K_i value of 70 nM (Entry 10, Table 1).

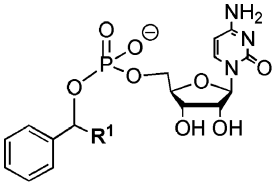
Diastereomers of the β-naphthyl derivative **3j** were also found to be highly potent sialyltransferase inhibitors (*e.g.* (*h*)-**3j**: $K_i = 130$ nM). Combining the positive effects of the 2-pyridyl and β-naphthyl systems, the quinolinyl compound **3l** was prepared. Although **3l** did not show the cumulative effects anticipated (*e.g.* (*l*)-**3l**: $K_i = 180$ nM), it was nonetheless another example of a potent aryl based sialyltransferase inhibitor. The pyrimidonyl derivative **3m** and α-naphthyl derivative **3k** also showed sialyltransferase inhibition, however the values are not in the sub-micro molar range of the other inhibitors described here.

Overall the configuration at the carbon atom bearing the CMP moiety appears to play a minor role in binding. Yet, in the case of the most promising inhibitors there is often at least a factor of four difference in activity between diastereomers (*c.f.*, (*R*)-**3b**/(*S*)-**3b**, Entries 3/4, Table 2). Thus, we embarked on a stereoselective synthesis of the most promising benzyl inhibitors, **3a**, the *m*-phenoxy derivative **3b**, and the *m*-trifluoromethyl derivative **3c**, in order: (a) assign the stereochemistry at the α-centre, (b) evaluate the role of stereochemistry on binding, and (c) access large quantities of stereochemically pure inhibitors for biological testing.

Asymmetric synthesis

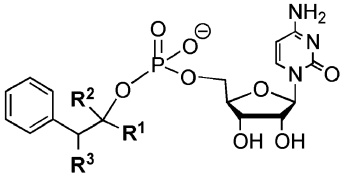
The preparation of the phosphonato sialyltransferase inhibitors described above involves condensation of the appropriate α-hydroxyphosphonate with cytidine phosphitamide, followed by oxidation with *tert*-butyl hydroperoxide, and base-catalysed cleavage of the cyanoethyl group. Subsequent removal of the phosphonate protecting groups, followed by

Table 1. Comparison of inhibition constants (K_i) of benzyl (**2**, **3a**) and phenylethyl (**4-8**) transition-state analogues containing carboxylate and phosphonate substituents against α(2-6)-sialyltransferase from rat liver.[a]



Benzyl Based Inhibitors

(**2**) $R^1 = CO_2^-$
(**5**) $R^1 = P(O)(O^-)_2$



Phenylethyl Based Inhibitors

(**3**) $R^1 = CO_2^-$; $R^2 = H$; $R^3 = H$
(**6**) $R^1 = P(O)(O^-)_2$; $R^2 = H$; $R^3 = H$
(**4**) $R^1 = CO_2^-$; $R^2 = CO_2^-$; $R^3 = H$
(**7**) $R^1 = P(O)(O^-)_2$; $R^2 = P(O)(O^-)_2$; $R^3 = H$
(**8**) $R^1 = P(O)(O^-)_2$; $R^2 = H$; $R^3 = Ph$

Entry	*Compound*				$K_i[\mu M]$	K_M/K_i
Benzyl Inhibitors		R^1				
1	(*S*)-**2**	CO_2^-			7 ± 2	7
2	(*R*)-**2**	CO_2^-			10 ± 2	5
3	(*S*)-**3a**	$P(O)(O^-)_2$			0.2 ± 0.02	230
4	(*R*)-**3a**	$P(O)(O^-)_2$			0.8 ± 0.2	58
Phenylethyl Inhibitors		R^1	R^2	R^3		
5	(*S*)-**4**	CO_2^-	H	H	23 ± 0.1	2
6	(*R*)-**4**	CO_2^-	H	H	15 ± 0.01	3
7	(*h*)-**5**	$P(O)(O^-)_2$	H	H	0.16 ± 0.08	288
8	(*l*)-**5**	$P(O)(O^-)_2$	H	H	1.1 ± 0.4	42
9	**6**	CO_2^-	CO_2^-	H	~ 10	~ 4[b]
10	**7**	$P(O)(O^-)_2$	$P(O)(O^-)_2$	H	12 ± 6	4[b]
11	(*h*)-**8**	$P(O)(O^-)_2$	H	Ph	1.5 ± 0.3	30
12	(*l*)-**8**	$P(O)(O^-)_2$	H	Ph	3.1 ± 0.6	15

[a]The affinity of the sialyl donor CMP-Neu5Ac (K_M) to α(2-6)-sialyltransferase from rat liver is 46 ± 7 μM [10].
[b]Mixed mode of inhibition.

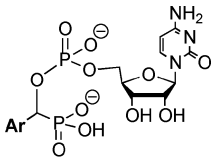
O/*N*-deacetylation, liberated the target inhibitors [10–14]. This method is highly practical and delivers the desired compounds in high yield, in just three steps from commercially available aryl aldehydes. The synthesis, however, generates a racemic mixture of α-hydroxyphosphonates in the first step, and eventually gives rise to a diastereomeric mixture of the target compounds, which require HPLC separation prior to biological evaluation.

To address this issue we have developed an asymmetric route to our sialyltransferase inhibitors starting from enantiomerically pure α-hydroxyphosphonates. Several methods for the synthesis of chiral, non-racemic α-hydroxyphosphonates have already been reported [22,23], as they are important bioactive molecules known to inhibit a range of enzymes, as well as attractive precursors to α-hydroxyphosphoryl isosteres of α-amino acids. The methods include chiral variations of either the Pudovik reaction [24] or the Abramov reaction [25], chemical [26] or enzymatic resolution [27], or employing catalytic asymmetric methods [23].

We have recently reported the synthesis of chiral, non-racemic α-hydroxyphosphonates [21] utilising the enantioselective oxaziridine (**17**) hydroxylation of phosphoryl stabilised anions introduced by Wiemer *et al.* [22,28]. Employing diallyl benzylphosphonates is a key element of the method, as they can be readily deprotected under neutral conditions [29] to afford the corresponding phosphonic acids in high enantiomeric excess (*ee*). In this manner, the requisite chiral, non-racemic α-hydroxyphosphonates (*S*)-**14a**, **b**, **n** (Scheme 5) and (*R*)-**14a**, **b**, **n** (not shown) were prepared stereoselectively from the corresponding achiral diallyl benzylphosphonates **16a-c** in good yield and high enantiomeric excess (96–98% *ee*) [21].

Employing our standard coupling methodology, each pure enantiomer of the particular α-hydroxyphosphonate derivative (*S*)-**14a**, **b**, **n** and (*R*)-**14a**, **b**, **n** was condensed with cytidine phosphitamide to afford after oxidation and base-catalysed cleavage, the fully protected forms of the inhibitors, (*S*)-**15a**, **b**, **n** and (*R*)-**15a**, **b**, **n** respectively. Pd-catalysed cleavage of the allyl phosphonate protecting groups under neutral conditions,

Table 2. Comparison of inhibition constants (K_i) of aryl transition-state analogues against α(2-6)-sialyltransferase.[a]



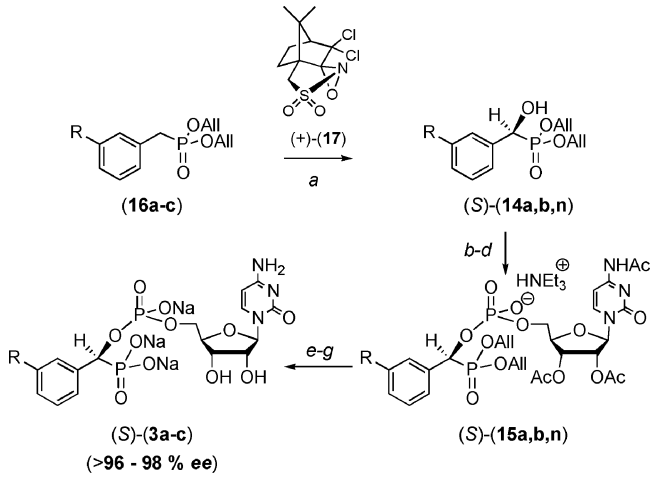
Aryl Based Inhibitors

Entry	*Cpd*	*Ar*	*K_i [μM]*	*K_M/K_i*
1	(*S*)-**3a**		0.2 ± 0.02	230
2	(*R*)-**3a**		0.8 ± 0.2	58
3	(*S*)-**3b**		0.3 ± 0.1	150
4	(*R*)-**3b**		0.07 ± 0.01	660
5	(*S*)-**3c**		0.22 ± 0.06	210
6	(*R*)-**3c**		0.25 ± 0.14	180
7	**3d**		0.3 ± 0.1	150
8	**3e**		0.6 ± 0.1	77
9	**3f**		0.4 ± 0.1	115
10	(*h*)-**3g**		0.07 ± 0.03	660
11	(*t*)-**3g**		0.19 ± 0.1	240
12	**3h**		0.36 ± 0.04	130
13	**3i**		>100[†]	–
14	**3j**		0.13 ± 0.03	350
15	**3k**		~1	46[c]
16	(*h*)-**3l**		0.6 ± 0.1	77
17	(*l*)-**3l**		0.18 ± 0.1	255
18	3m		3 ± 1	15

[a]The affinity of the sialyl donor CMP-Neu5Ac (K_M) to α(2-6)-sialyltransferase from rat liver is 46 ± 7μM [10].
[b]Mixed mode of inhibition.
[c]Mode of inhibition unclear.

followed by *O*/*N*-deacetylation and ion exchange gave the diastereomerically pure sialyltransferase inhibitors (*S*)-**3a**–**c** and (*R*)-**3a**–**c** (>96% *de*) as sodium salts (Scheme 5).

In this way, the absolute stereochemistry of the inhibitors could be assigned and the binding affinity of the various stereoisomers assessed. By comparing the inhibition values for the phenyl derivatives (*S*)-**3a**–**c** with those of (*R*)-**3a**–**c**, no consistent trend emerged regarding the effect of the nature of the α-phosphoryl stereogenic centre. For instance, the trifluoromethyl-substituted compounds (*S*)-**3c** and (*R*)-**3c** exhibited almost identical inhibition values (Entries 7/8). In the case of the simple benzyl-derived inhibitors (*S*)-**3a**/(*R*)-**3a**, the diastereomer with the (*S*)-configuration at the α-centre is four



a: R = H; **b**: R = OPh; **c**/**n**: R = CF_3

Scheme 5. Asymmetric synthesis of potent benzyl sialyltransferase inhibitors (*S*)-(**3a-c**). *Reagents and Conditions:* (a) 1.5 eq. NaHMDS, 2 eq. (+)-(**17**), THF, –78°C, 3 h; (b) 1.5 eq. (**10**), 2.0 eq. tetrazole, CH_2Cl_2, RT, 3 h; (c) 1.5 eq. *t*-BuOOH, RT, 1 h; (d) 50 eq. NEt_3, RT, 18 h; (e) 20 mol. % $Pd(Ph_3)_4$, 10.0 eq. dimedone, THF, RT, 18 h; (f) 25% NH_3-H_2O, RT, 18 h; (g) IR 120 Na^+.

times more potent than the (*R*)-configured diastereomer. On the other hand however with the *m*-phenoxybenzyl-derived inhibitors (*S*)-**3b**/(*R*)-**3b**, the trend is reversed, with the (*R*)-configured diastereomer showing four-fold greater activity. (*cf* Entries 5/6, Table 1). It appears therefore that stereoselective formation of the inhibitors and separate biological evaluation of the stereoisomers is important, and may result in significant differences in the level of enzyme inhibition.

Materials and methods

General details

Unless specified otherwise, all reactions were performed under an inert atmosphere of nitrogen with dry, freshly distilled solvents under anhydrous conditions and monitored by TLC using plastic plates coated with Merck Silica Gel 60 F254 and visualised using either UV light (254 or 366 nm) or a molybdenum staining reagent (a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulphuric acid (400 mL)).

Starting materials and reagents

The cytidine phosphitamide **10** [30], 6-pyrimidone-4 carboxaldehyde hydrochloride [31,32], the enantiomerically pure α-hydroxyphosphonates (*S*)-**14a**–**c** and (*R*)-**14a**–**c** [21], and the sialyltransferase inhibitors (*S*)-**2**/(*R*)-**2**, (*S*/*R*)-**3a**, and (*S*)-**4**/(*R*)-**4**,were prepared as described previously [13]. The following compounds were obtained from Aldrich, Fluka or Acros and used as supplied: *p*-acetamidobenzaldehyde, *p*-nitrobenzaldehyde, *p*-amylbenzaldehyde,

α/β-naphthaldehyde, quinoline-2-carboxaldehyde and benzaldehyde.

Purification methods

All compounds were purified by flash chromatography (FC) as described by Still *et al.* [33] at a pressure of 0.3–0.4 bar using Merck Silica Gel 60 (particle size 40–63 m) and the yields given refer to chromatographically and spectroscopically (^{1}H NMR) homogenous material. MPLC was performed at a pressure of 5–10 bar on silica gel columns LiChroprep Si 60 (Merck, 15–25 μm, 28 × 2.5 cm). Preparative HPLC was performed with a Shimadzu LC8A preparative pump and a Rainin Dynamax UV 1 detector at 260 nm; columns: (A) Eurospher 100-C18 (Knauer, 7 μm, 250 × 16 mm), (B) Eurospher 100-C18 (Knauer, 7 μm, 250 × 20 mm), (C) LiChrospher 100 RP18 (Merck, 7 μm, 250 × 25 mm). Mixtures of acetonitrile and 0.05 M triethylammonium bicarbonate (TEAB) (pH 7.2–7.5) were used as mobile phase. Analytical HPLC was performed on a Merck-Hitachi system with a L 7200 autosampler and a L 4000 UV detector; column: Eurosphere 100-C18 (Knauer GmbH, 5 μm, 250 × 4 mm).

Analytical methods

NMR spectra were recorded on a Bruker AC 250 Cryospec, a Bruker DRX 600, or a JEOL JNM-GX 400 instrument, where the solvent ^{1}H and ^{13}C signals, δ_H 7.24 for residual $CHCl_3$ and δ_C 77.0 for $CDCl_3$, δ_H 3.31 and δ_C 49.0 for D_4-MeOH, and δ_H 4.63 for D_2O (a drop of D_4-MeOH was added to D_2O for referencing ^{13}C spectra), were used as internal references. For ^{31}P NMR spectra, phosphoric acid was used as an external reference. Signal assignments are based on a combination of H,H-COSY, HMQC, ROESY and SED data. Matrix-assisted laser desorption ionisation mass spectra (MALDI-MS) were recorded on a Kratos Kompact Maldi 2, using 2,5-dihydroxybenzoic acid (DHB) or 6-azathiothymine (ATT) as matrices. FAB-mass spectra were measured on a Finnigan MAT 312/AMD 5000 (70 eV, 70°C). Optical rotations were measured on a Büchi polar monitor in a 1 dm cell at 22°C. Elemental analyses were measured on a Heraeus CHN—O—Rapid. Melting points are reported in degrees Celsius (uncorrected).

Sialyltransferase assay

Measurement of the inhibition constants K_i of the various transition-state analogues against α(2-6)-sialyltransferase from rat liver is based on a previously reported assay [10].

Racemic synthesis of phenylethyl (2-6)sialyltransferase inhibitors

Protocol A: As described for previous syntheses of sialyltransferase inhibitors [12], the requisite α-hydroxyphosphonates were obtained by dissolving the appropriate aldehyde (1 eq.) (or acid chloride for bis-phosphonate **12b**) in a small amount of CH_2Cl_2 and reacting with either dibenzyl or diallyl protected phosphonic acid diester (2 eq.) in the presence of a few drops of NEt_3. The purified racemic α-hydroxyarylphosphonates (1 eq.) obtained after isolation were then dissolved in CH_2Cl_2 along with cytidine phosphitamide **10** (1.5 eq.) and evaporated to dryness. The remaining foam was dissolved in dry CH_2Cl_2, and 1-*H* tetrazole (2 eq.) was added. After stirring the reaction for 3 h, an anhydrous solution of *t*-butyl hydroperoxide (1.5 eq.) was added, followed, after an additional hour, by the addition of NEt_3 (50 eq.). After a further 18 h of stirring, the reaction mixture was concentrated at 20°C and the residue purified by silica gel flash chromatography.

O-Debenzylation or *O*-deallylation, followed by *O*/*N*-deacetylation was performed as described previously [12,14]. The diastereomeric mixture of inhibitor was separated by HPLC, and each of the purified diastereomers converted to their sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give the desired sialyltransferase inhibitors, in good overall yield.

Trisodium cytidin-5′-yl-[2-phenyl-1-phosphonatoethyl]-phosphate (**5**)

From diallyl α-hydroxyphosphonate **12a** according to protocol A: deallylation of the triethylammonium salt **13a**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**5**. The diastereomers (*h*)-**5** and (*l*)-**5**, were separated by HPLC, converted to their sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give a mass of 20 mg (combined mass of diastereomers), 31% yield from aldehyde. (*h*)-**5**: HPLC (prep. RP-18, 0–10 mn isocratic 5% CH_3CN, 10–60 mn linear gradient: 5–15% CH_3CN, 15 mL/mn flow): t_R = 28.5 min; ^{1}H NMR (250 MHz, D_2O): δ 2.73–3.96 (m, 7H, 2′-H, 3′-H, 4′-H, 5′a,b-H, ArCH_2), 4.24 (dd, 1H, 1″-H), 5.67 (d, J(1′,2′) = 4.2 Hz, 1H, 1′-H), 5.87 (d, J(5,6) = 7.7 Hz, 1H, 5-H), 7.04–7.19 (m, 5H, ArH), 7.59 (d, ^3J(6,5) = 7.7 Hz, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT) m/z = 507 [M-3Na+2H]$^-$; 573.3 for $C_{17}H_{20}N_3Na_3O_{11}P_2$. (*l*)-**5**: HPLC (prep. RP-18, 0–10 mn isocratic 5% CH_3CN, 10–60 mn linear gradient: 5–15% CH_3CN, 15 mL/mn flow): t_R = 30.6 min; ^{1}H NMR (250 MHz, D_2O): δ 2.72–3.98 (5 m, 7H, 2′-H, 3′-H, 4′-H, 5′a,b-H, ArCH_2), 4.25 (m, 1H, 1″-H), 5.64 (d, J(1′,2′) = 4.2 Hz, 1H, 1′-H), 5.88 (d, J(5,6) = 7.7 Hz, 1H, 5-H), 7.02–7.18 (m, 5H, ArH), 7.61 (d, J(6,5) = 7.7 Hz, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT) m/z = 507 [M-3Na+2H]$^-$; 573.3 for $C_{17}H_{20}N_3Na_3O_{11}P_2$.

Trisodium cytidin -5′-yl-[1,1-dicarboxylato-2-phenylethyl]-phosphate (**6**)

From dibenzyl benzyltartronate **9b** according to protocol A: debenzylation and deacetylation of the protected intermediate **11b** by treatment with aqueous lithium hydroxide solution (0.1 mL, 1 M) in dioxane (2 mL), conversion to the sodium salt

form by ion-exchange (IR 120 Na+), precipitation from EtOH and lyophilisation from water gave **6** (6 mg, 48% yield from alcohol). TLC: $R_f = 0.05$ (EtOAc-MeOH); ^{1}H NMR (600 MHz, D_2O): δ 3.45 (bs, 2H, 2″a-H,2″b-H), 3.98 (m, 1H, 5′a-H), 4.03–4.08 (m, 4H, 2′-H, 3′-H, 4′-H, 5′b-H), 5.77 (d, $^3J(1',2') = 3.4$ Hz, 1H, 1′-H), 5.93 (d, $^3J(5,6) = 7.5$ Hz, 1H, 5-H), 7.24 (bs, 2H, ArH), 7.06 (d, $^3J(m, p) = 7.2$ Hz, 1H, *p*-ArH), 7.10 (dd, $^3J(m, p) = 7.2$ Hz, $^3J(o, m) = 7.4$ Hz, 1H, *m*-ArH), 7.27 (d, $^3J(o, m) = 7.4$ Hz, 1H, *o*-ArH), 7.82 (d, $^3J(5,6) = 7.5$ Hz, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT) m/z = 590 ([M-3Na+2K]$^-$, 40%), 568 ([M-2Na + 2K-CO_2-H]$^-$, 100%); MALDI-MS (positive mode, matrix: DHB) m/z = 582 ([M + H]$^+$, 20%), 560 ([M-Na + 2H]$^+$, 30%), 538 ([M-2Na + 3H]$^+$, [M-CO_2 + H]$^+$, 100%), 516 ([M-3Na + 4H]$^+$, [M-CO_2-Na + 2H]$^+$, 80%), 494 ([M-CO_2-2Na + 3H]$^+$, 45%); 581.0 for $C_{19}H_{19}N_3Na_3O_{12}P$.

Pentasodium cytidin-5′-yl-[2-phenyl-1,1-diphosphonatoethyl]-phosphate (**7**)

From tetraallyl α-hydroxybisphosphonate **12b** (prepared from phenacetylchloride) according to protocol A: deallylation of the triethylammonium salt **13b**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**7**, which was converted to the sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give (S/*R*)-**7** (16 mg, 9% yield from acid chloride). ^{1}H NMR (250 MHz, D_2O): δ 3.31–3.42 (t, ^3J(H,P) = 11.8 Hz, 2H, $ArCH_2$), 4.01–4.10 (m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 5.72 (d, 1H, 1′-H), 5.86 (d, $^3J(5,6) = 7.5$ Hz, 1H, 5-H), 6.99–7.04 (m, 3H, ArH), 7.34–7.40 (m, 2H, ArH), 7.82 (d, $^3J(6,5) = 7.5$ Hz, 1H, 6-H).

Trisodium cytidin-5′-yl-[2,2-diphenyl-1-phosphonatoethyl]-phosphate (**8**)

From diallyl α-hydroxyphosphonate **12c** according to protocol A: deallylation of the triethylammonium salt **13c**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**8**. The diastereomers (*h*)-**8** and (*l*)-**8**, were separated by HPLC, converted to their sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give 18 mg (combined mass of diastereomers), 27% yield from aldehyde. (*h*)-**8**: HPLC (prep. RP-18, 0–10 mn isocratic 5% CH_3CN, 10–60 mn linear gradient: 5–15% CH_3CN, 15 mL/mn flow): $t_R = 34.2$ min; ^{1}H NMR (250 MHz, D_2O): δ 3.52–3.98 (3 m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 4.42–4.53 (m, 1H, 2″-H), 4.83–4.97 (dd, 1H, 1″-H), 5.69 (d, $^3J(1',2') = 3.7$ Hz, 1H, 1'-H), 5.91 (d, $^3J(5,6) = 7.4$ Hz, 1H, 5-H), 6.97–7.14 (m, 6H, ArH), 7.23–7.49 (m, 4H, ArH), 7.71 (d, $^3J(5,6) = 7.4$ Hz, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT) m/z = 582 [M-3Na + 2H]$^-$; 649.4 for $C_{23}H_{24}N_3Na_3O_{11}P_2$. (*l*)-**8**: HPLC (prep. RP-18, 0–10 mn isocratic 5% CH_3CN, 10–60 mn linear gradient: 5–15% CH_3CN, 15 mL/mn flow): $t_R = 36.7$ mn; ^{1}H NMR (250 MHz, D_2O): δ 3.52–3.98 (3m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 4.38–4.43 (m, 1H, 2″-H), 4.72–4.98 (dd, 1H, 1″-H), 5.66 (d, $^3J(1',2') = 2.1$ Hz, 1H, 1′-H), 5.94 (d, $^3J(5,6) = 7.3$ Hz, 1H, 5-H), 6.97–7.14 (m, 6H, ArH), 7.23–7.49 (m, 4H, ArH), 7.70 (d, $^3J(6,5) = 7.3$ Hz, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT) m/z = 582 [M-3Na+2H]$^-$; 649.4 for $C_{23}H_{24}N_3Na_3O_{11}P_2$.

Racemic synthesis of arylmethyl α(2-6)sialyltransferase inhibitors

Trisodium cytidin-5′-yl-[(3-phenoxy)phenyl-phosphonatomethyl]-phosphate (**3b**)

From diallyl α-hydroxyphosphonate ((*S*/*R*)-**14b**)[1] according to protocol A: deallylation of the triethylammonium salt **15b**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**3b**. The diastereomers (*h*)-**3b** and (*l*)-**3b**, were separated by HPLC, converted to their sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give a mass of 18 mg (combined mass of diastereomers), 31% yield from aldehyde. (*h*)-**3b**: HPLC (prep. RP-18, 0–40 mn linear gradient: 5–11.5% CH_3CN, 0.05 M TEAB, 16 mL/mn flow): $t_R = 32$ mn; ^{1}H NMR (D_2O, 600 MHz): δ 3.67 (m, 1H, 3′-H), 3.71 (m, 1H, 5′a-H), 3.75 (m, 1H, 5′b-H), 3.91 (m, 1H, 2′-H), 3.92 (m, 1H, 4′-H), 5.01–5.11 (m, 1H, CHP), 5.78 (d, $J(1',2') = 4.5$ Hz, 1H, 1′-H), 5.91 (d, $J(5,6) = 7.5$ Hz, 1H, 5-H), 6.64–7.30 (m, 9H, ArH), 7.65 (d, $^3J(6,5) = 7.5$ Hz, 1H, 6-H);^{13}C NMR (D_2O, 151 MHz): δ 63.8 (C5′), 68.8 (C3′), 73.8 (C2′), 75.8 (^1J(C,P) = 159 Hz, CHP), 82.3 (C4′), 88.3 (C1′), 96.1 (C5), 117.1, 117.5, 118.1, 122.6, 123.2, 128.9, 129.5 (ArCH), 140.2 (C6), 140.6 (ArC), 155.6 (C4), 156.0, 157.0 (Ph—O—C) 165.5 (C2); ^{31}P NMR (D_2O, 243 MHz): δ 1.78 (d, ^3J(P,P) = 32 Hz, $P(O)O_3$), 14.26 (d, ^3J(P,P) = 32 Hz, $P(O)O_2$); MALDI-MS (negative mode, matrix: THAP) m/z = 584 ([M-3Na+2H]$^-$, 651.04 for $C_{22}H_{22}N_3Na_3O_{12}P_2$. (*l*)-**3b**: HPLC (prep. RP-18, 0–40 mn linear gradient: 5–11.5% CH_3CN, 0.05 M TEAB, 16 mL/mn flow): $t_R = 33$ mn; ^{1}H NMR (D_2O, 600 MHz): δ 3.67 (m, 1H, 5′a-H), 3.82 (m, 2H, 3′-H, 5′b-H), 3.90 (m, 1H, 2′-H), 3.92 (m, 1H, 4′-H), 4.96–5.05 (m, 1H, CHP), 5.73 (d, $J(1',2') = 3.7$ Hz, 1H, 1′-H), 5.94 (d, $J(5,6) = 7.5$ Hz, 1H, 5-H), 6.79-6.80 (m, 1H, Ph-6-H), 6.90–6.91 (m, 2H, OPh-*ortho*-H), 7.040–7.08 (m, 2H, OPh-*para*-H, Ph-2-H), 7.20-7.22 (m, 2H, Ph-4-H, Ph-5-H), 7.25–7.28 (m, 2H, OPh-*meta*-H), 7.58 (d, $^3J(6,5) = 7.5$ Hz, 1H, 6-H);^{13}C NMR (D_2O, 151 MHz): δ 63.8 (C5′), 68.9 (C3′), 74.9 (C2′), 77.2 (^1J(C,P) = 159 Hz, CHP), 82.8 (C4′), 89.4 (C1′), 96.9 (C5), 118 (Ph-C6), 119 (OPh-C2, OPh-C6), 119.5 (Ph-C2), 124 (Ph-C4), 124.5 (OPh-C4), 130 (Ph-C5), 131 (OPh-C3, OPh-C5), 141 (C6); ^{31}P NMR (D_2O, 243 MHz): δ 2.34 (d, ^3J(P,P) = 30.5 Hz, $P(O)O_3$), 13.98 (d, ^3J(P,P) = 30.5 Hz, $P(O)O_2$); MALDI-MS (negative mode, matrix: THAP) m/z = 584 ([M-3Na+2H]$^-$, 651.04 for $C_{22}H_{22}N_3Na_3O_{12}P_2$.

Trisodium cytidin-5′-yl-[(3-trifluoromethyl)phenyl-phosphonatomethyl]-phosphate (**3c**)

From dibenzyl α-hydroxyphosphonate (*S*/*R*)-**14c** according to protocol A: debenzylation of the triethylammonium salt **15c**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**3c**. The diastereomers (*h*)-**3c** and (*l*)-**3c**, were separated by HPLC, converted to their sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give a mass of 11 mg (combined mass of diastereomers), 33% yield from aldehyde. (*h*)-**3c**: HPLC (prep. RP-18, 0–5 mn: isocratic 1% CH_3CN, 5–30 mn linear gradient: 1–10% CH_3CN, 0.05 M TEAB, 10 mL/mn flow): t_R = 23.2 mn; ^{1}H NMR (D_2O, 250 MHz): δ 3.51–3.92 (m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 5.13 (dd, $^3J(1'',P)$ = 14.1 Hz, $^2J(1'',P)$ = 10.2 Hz, 1H, CHP), 5.70 (d, $J(1',2')$ = 4.4 Hz, 1H, 1′-H), 6.03 (d, $J(5,6)$ = 7.8 Hz, 1H, 5-H), 7.39-7.62 (m, 4H, ArH), 7.82 (d, $^3J(6,5)$ = 7.8 Hz, 1H, 6-H); ^{13}C NMR (D_2O/D_4-MeOH, 63 MHz): δ 65.2 (C3′), 70.1 (C2′), 74.0 (^1J(C,P) = 173.6 Hz, CHP), 75.2 (C5′), 83.5 (J(C,P) = 8.9 Hz, C4′), 90.1 (C1′), 97.3 (C5), 125.0, 125.4, 129.7, 132.2 (ArCH), 139.9 (s, ArC), 142.1 (C6), 158.5 (C2), 167.0 (C4); ^{31}P NMR (D_2O, 162 MHz): δ 4.00 (d, ^3J(P,P) = 32.4 Hz, $P(O)O_3$), 16.50 (d, ^3J(P,P) = 32.4 Hz, $P(O)O_2$); MALDI-MS (negative mode, matrix: ATT) m/z = 562 (M-3Na+2H)$^-$, 627.4 for $C_{17}H_{17}F_3N_3Na_3O_{11}P_2$. (*l*)-**3c**: HPLC (prep. RP-18, 0–5 mn: isocratic 1% CH_3CN, 5–30 mn linear gradient: 1-10% CH_3CN, 0.05 M TEAB, 10 mL/mn flow): t_R = 24.2 mn; ^{1}H NMR (D_2O, 250 MHz): δ 3.51–3.92 (m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 5.11 (dd, $^3J(1'',P)$ = 14.1 Hz, $^2J(1'',P)$ = 10.3 Hz, 1H, CHP), 5.61 (d, $J(1',2')$ = 3.4 Hz, 1H, 1′-H), 5.99 (d, $J(5,6)$ = 7.8 Hz, 1H, 5-H), 7.34–7.62 (m, 4H, ArH), 7.69 (d, $^3J(6,5)$ = 7.8 Hz, 1H, 6-H); ^{13}C NMR (D_2O, 63 MHz): δ 65.0 (C3′), 70.1 (C2′), 76.1 (C5′), 78.0 (^1J(C,P) = 152.9 Hz, CHP), 83.9 (J(C,P) = 9.3 Hz, C4′), 91.0 (C1′), 98.0 (C5), 125.8, 125.9, 130.3, 132.9 (ArCH), 141.3 (s, ArC), 142.9 (C6), 159.2 (C2), 167.8 (C4); ^{31}P NMR (D_2O, 162 MHz): δ 4.40 (d, ^3J(P,P) = 32.4 Hz, $P(O)O_3$), 16.03 (d, ^3J(P,P) = 32.4 Hz, $P(O)O_2$); MALDI-MS (negative mode, matrix: ATT) m/z = 562 [M-3Na+2H]-, 627.4 for $C_{17}H_{17}F_3N_3Na_3O_{11}P_2$.

Trisodium cytidin-5′-yl-[(4-acetamido)phenyl-phosphonatomethyl]-phosphate (**3d**)

From dibenzyl α-hydroxyphosphonate **14d** according to protocol A: debenzylation of the triethylammonium salt **15d**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**3d**. The diastereomers (*h*)-**3d** and (*l*)-**3d**, were separated by HPLC, converted to their sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give a mass of 15 mg (combined mass of diastereomers), 31% yield from aldehyde. (*h*)-**3d**: HPLC (prep. RP-18, 0.1% CH_3CN, 0.05 M TEAB, 10 mL/mn flow): t_R = 14.7 mn; ^{1}H NMR (600 MHz, D_2O): δ 2.06 (s, 3H, Ac), 3.66 (m, 1H, 3′-H), 3.81-3.94 (m, 3H, 2′-H, 5′a,b-H), 3.97 (m, 1H, 4′-H), 5.06 (dd, $^3J(1'',P)$ = 13.6 Hz, $^2J(1'',P)$ = 10.1 Hz, 1H, CHP), 5.66 (d, $J(1',2')$ = 3.4 Hz, 1H, 1′-H), 5.94 (d, $J(5,6)$ = 7.7 Hz, 1H, 5-H), 7.22–7.35 (m, 4H, ArH), 7.82 (d, $^3J(6,5)$ = 7.7 Hz, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT) m/z = 551 [M-3Na+2H]$^-$; 616.4 for $C_{18}H_{21}N_4Na_3O_{12}P_2$. (*l*)-**3d**: HPLC (prep. RP-18, 0.1% CH_3CN, 0.05M TEAB, 10 mL/mn flow): t_R = 16.4 mn; ^{1}H NMR (600 MHz, D_2O): δ 2.08 (s, 3H, Ac), 3.54–3.58 (m, 1H, 3′-H), 3.74–3.92 (m, 3H, 2′-H, 5′a,b-H), 3.97 (m, 1H, 4′-H), 5.11 (dd, $^3J(1'',P)$ = 12.8 Hz, $^2J(1'',P)$ = 10.5 Hz, 1H, 1″-H), 5.78 (d, $J(1',2')$ = 3.4 Hz, 1H, 1′-H), 5.93 (d, $J(5,6)$ = 7.7 Hz, 1H, 5-H), 7.30–7.39 (m, 4H, ArH), 7.59 (d, $^3J(6,5)$ = 7.7 Hz, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT) m/z = 551 [M-3Na+2H]$^-$; 616.4 for $C_{18}H_{21}N_4Na_3O_{12}P_2$.

Trisodium cytidin-5′-yl-[(4-nitro)phenyl-phosphonatomethyl]-phosphate (**3e**)

From diallyl α-hydroxyphosphonate **14e** according to protocol A: deallylation of the triethylammonium salt **15e**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**2e**, which was further purified by HPLC, converted to the sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give (S/*R*)-**2e** (23 mg, 28% yield from aldehyde). HPLC (prep. RP-18, 0–10 mn isocratic 2% CH_3CN, 0.05 M TEAB, 10 mL/mn flow): t_R = 9.4 mn; ^{1}H NMR (250 MHz, D_2O): δ 3.69–4.13 (m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 5.11 (dd, $^3J(1'',P)$ = 15.4 Hz, $^2J(1'',P)$ = 9.8 Hz, 1H, 1″H), 5.60/5.66 (2d, $J(1',2')$ = 3.5/4.2 Hz, 1H, 1′-H), 5.89/5.93 (2d, $J(5,6)$ = 7.7 Hz, 1H, 5-H), 7.45–7.51 (m, 2H, ArH), 7.55/7.74 (d, $^3J(6,5)$ = 7.7 Hz, 1H, 6-H), 8.01–8.14 (m, 2H, ArH); MALDI-MS (negative mode, matrix: ATT) m/z = 539 [M-3Na+2H]$^-$; 604.3 for $C_{16}H_{17}N_4Na_3O_{13}P_2$.

Trisodium cytidin-5′-yl-[(4-amyl)phenyl-phosphonatomethyl]-phosphate (**3f**)

From diallyl α-hydroxyphosphonate **14f** according to protocol A: deallylation of the triethylammonium salt **15f**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**3f**, which was further purified by HPLC, converted to the sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give (S/*R*)-**3f** (17 mg, 42% yield from aldehyde). HPLC (prep. RP-18, 0–10 mn isocratic 1% CH_3CN, 0.05 M TEAB, 10 mL/mn flow): t_R = 45 mn; ^{1}H NMR (250 MHz, D_2O): δ 0.69 (m, 3H, 5″-H), 0.95–1.15 (m, 4H, 3″-H, 4″-CH_2), 1.19–1.42 (m, 2H, 2″-H), 2.27-2.44 (m, 2H, 1″-H), 3.45–3.89 (m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 4.98 (dd, $^3J(1'',P)$ = 12.7 Hz, $^2J(1'',P)$ = 9.9 Hz, 1H, 1″H), 5.54/5.66 (2d, $J(1',2')$ = 3.5/4.3 Hz, 1H, 1′-H), 5.86 (2d, $J(5,6)$ = 7.7 Hz, 1H, 5-H), 6.78–7.25 (m, 4H, ArH), 7.29/7.47 (d, $^3J(6,5)$ = 7.7 Hz, 1H, 6-H); MALDI-MS (negative mode, matrix: ATT)

m/z = 546 [M-3Na-H_2O+2H]$^-$, 564 [M-3Na+2H]$^-$; 629.4 for $C_{21}H_{28}N_3Na_3O_{11}P_2$.

Trisodium cytidin-5′-yl-[phosphonato-(pyridin-2-yl)methyl]-phosphate (**3g**)

From diallyl α-hydroxyphosphonate **14g** according to protocol A: deallylation of the triethylammonium salt **15g**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and O/N-deacetylation gave (S/*R*)-**3g**. The diastereomers (*h*)-**3g** and (*l*)-**3g**, were separated by HPLC, converted to their sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give 40 mg (combined mass of diastereomers), 34% yield from aldehyde. (*h*)-**3g**: HPLC (prep. RP-18, 0–10 mn isocratic 1% CH_3CN, 10–60 mn linear gradient: 1–10% CH_3CN, 15 mL/mn flow): t_R = 12.2 min; ^{1}H NMR (250 MHz, D_2O): δ 3.40–3.53 / 3.72–3.57 (m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 4.88–4.97 (dd, $^3J(1'',P)$ = 13.7 Hz, $^2J(1'',P)$ = 9.5 Hz, 1H, 1″-H), 5.68 (d, $^3J(1',2')$ = 5 Hz, 1H, 1′-H), 5.87 (d, $^3J(5,6)$ = 7.6 Hz, 1H, 5-H), 7.07–7.12 (m, 1H, ArH), 7.46–7.59 (m, 2H, ArH), 7.56 (d, $^3J(6,5)$ = 7.6 Hz, 1H, 6-H), 8.18–8.20 (m, 1H, ArH); MALDI-MS (negative mode, matrix: ATT) m/z = 494 [M-3Na+2H]$^-$; 560.2 for $C_{15}H_{17}N_4Na_3O_{11}P_2$. (*l*)-**3g**: HPLC (prep. RP-18, 0–10 mn isocratic 1% CH_3CN, 10–60 mn linear gradient: 1–10% CH_3CN, 15 mL/mn flow): t_R = 13.8 min; ^{1}H NMR (250 MHz, D_2O): δ 3.59–3.64/3.73–3.85 (m, 5H, 1′-H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 4.86–4.95 (dd, $^3J(1'',P)$ = 13.8 Hz, $^2J(1'',P)$ = 9.5Hz, 1H, 1″-H), 5.58 (d, $^3J(1',2')$ = 3.6Hz, 1H, 1′-H), 5.87 (d, $^3J(5,6)$ = 7.6 Hz, 1H, 5-H), 6.02–7.05 (m, 1H, ArH), 7.43–7.50 (m, 2H, ArH), 7.45 (d, $^3J(6,5)$ = 7.6Hz, 1H, 6-H), 8.13–8.18 (m, 1H, ArH); MALDI-MS (negative mode, matrix: ATT) m/z = 494 [M-3Na+2H]$^-$; 560.2 for $C_{15}H_{17}N_4Na_3O_{11}P_2$.

Trisodium cytidin-5′-yl-[phosphonato-(pyridin-4-yl)methyl]-phosphate (**3h**)

From diallyl α-hydroxyphosphonate **14h** according to protocol A: deallylation of the triethylammonium salt **15h**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and O/N-deacetylation gave (S/*R*)-**3h**, which was further purified by HPLC, converted to the sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give (S/*R*)-**3h** (34mg, 46% yield from aldehyde). HPLC (prep. RP-18, 0–10 mn isocratic 1% CH_3CN, 10–60 mn linear gradient: 1–10% CH_3CN, 15 mL/mn flow): t_R = 13 mn; ^{1}H NMR (250 MHz, D_2O): δ 3.48–3.91 (3m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 4.71-4.92 (2dd, $^3J(1'',P)$ = 14.2 Hz, $^2J(1'',P)$ = 10.4 Hz, 1H, 1″-H), 5.59 (d, $^3J(1',2')$ = 3.1 Hz, 0.5H, 1′-H), 5.65 (d, $^3J(1',2')$ = 3.4Hz, 0.5H, 1′-H), 5.82–5.85 (2d, $^3J(5,6)$ = 7.4, 1H, 5-H), 7.24 (bs, 2H, ArH), 7.48 (d, $^3J(5,6)$ = 7.4 Hz, = 0.5H, 6-H), 7.53 (d, $^3J(5,6)$ = 7.4 Hz, 0.5H, 6-H), 8.05–8.19 (m, 2H, ArH); MALDI-MS (negative mode, matrix: ATT) m/z = 494 [M-3Na+2H]$^-$; 560.2 for $C_{15}H_{17}N_4Na_3O_{11}P_2$.

Trisodium cytidin-5′-yl-[(N-methylpyridinium-4-yl)-phosphonatomethyl]-phosphate (**3i**)

The triethylammonium salt 15 h (100 mg, 0.12 mmol) was dissolved in acetone (1 mL), followed by the drop wise addition of methyliodide (70 mg, 0.5 mmol) and stirred for 2 h, concentrated, purified by FC (silica gel: gradient EtOAc-MeOH +1% Et_3N, 5:1 to 1:50), and then 20 mg (0.03 mmol) of this material was deallylated, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and O/N-deacetylation to give (S/*R*)-3i, which was converted to the sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give (S/*R*)-3i (11 mg, 59% yield over 2 steps). ^{1}H-NMR (250 MHz, D_2O): δ 3.92–4.00 (m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 4.07/4.09 (2s, 3H, CH_3-N^+-H), 4.98–5.12 (2dd, 1H, 1″-H), 5.68 (d, $^3J(1',2')$ = 3.1Hz, 0.5H, 2′-H), 5.71 (d, $^3J(2',1')$ = 2.9 Hz, 0.5H, 2′-H), 5.79–5.86 (2d, $^3J(5,6)$ = 7.6Hz, 1H, 5-H), 7.61 (d, $^3J(6,5)$ = 7.6 Hz, 0.5H, 6-H), 7.68 (d, $^3J(6,5)$ = 7.6 Hz, 0.5H, 6-H), 7.75–7.78 (m, 2H, ArH), 8.33–8.38 (m, 2H, ArH); (MALDI-MS, negative mode, matrix ATT) m/z = 507 [M-2Na+H]$^-$; 552.3 for $C_{16}H_{20}N_4Na_2O_{11}P_2$.

Trisodium cytidin-5′-yl-[(naphthalin-2-yl)-phosphonatomethyl]-phosphate (**3j**)

From diallyl α-hydroxyphosphonate **14j** according to protocol A: deallylation of the triethylammonium salt **15j**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and O/N-deacetylation gave (S/*R*)-**3j**, which was further purified by HPLC, converted to the sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give (S/*R*)-**3j** (19 mg, 36% yield from aldehyde). HPLC (prep. RP-18, 0–10 mn isocratic 5% CH_3CN, 10–60 mn linear gradient: 2–20% CH_3CN, 15 mL/mn flow): t_R = 24 mn; ^{1}H NMR (250 MHz, D_2O): δ 2.98–3.82 (4m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 4.99–5.12 (2dd, 1H, 1″-H), 5.19 (d, $^3J(1',2')$ = 1.9 Hz, 0.5H, 1′-H), 5.31 (d, $^3J(1',2')$ = 3.4 Hz, 0.5H, 1′-H), 5.64 (2d, $^3J(5,6)$ = 7.5 Hz, 1H, 5-H), 7.15 (d, $^3J(6,5)$ = 7.5 Hz, 0.5H, 6-H), 7.18–7.28 (m, 2.5H, 6-H, ArH), 7.49–7.68 (m, 5H, ArH); MALDI-MS (negative mode, matrix: ATT) m/z = 542 [M-3Na+2H]$^-$, 564.7 [M-2Na+H]$^-$; 609.3 for $C_{20}H_{20}N_3Na_3O_{11}P_2$.

Trisodium cytidin-5′-yl-[(naphthalin-1-yl)-phosphonatomethyl]-phosphate (**3k**)

From diallyl α-hydroxyphosphonate **14k** according to protocol A: deallylation of the triethylammonium salt **15k**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and O/N-deacetylation gave (S/*R*)-3k. The diastereomers (*h*)-**3k** and (*l*)-**3k**, were separated by HPLC, converted to their sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give 19 mg (combined mass of diastereomers), 22% yield from aldehyde. (*h*)-**3k**: HPLC (prep. RP-18, 0–10 mn isocratic 2% CH_3CN, 10–60 mn linear gradient: 1–15% CH_3CN, 15 mL/mn flow): t_R = 27.2 mn; ^{1}H NMR

(250 MHz, D_2O): δ 2.98–3.76 (3m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 5.47 (d, $^3J(1',2') = 4.6$ Hz, 1H, 1′-H), 5.53 (d, $^3J(5,6) = 7.8$ Hz, 1H, 5-H), 5.91 (dd, $^2J(1'',P) = 12.8$ Hz, $^3J(1'',P') = 11.3$ Hz, 1H, 1″-H), 7.29-7.39 (m, 3H, ArH), 7.45 (d, $^3J(6,5) = 7.8$ Hz, 1H, 6-H), 7.60–7.73 (m, 3H, ArH), 8.00–8.03 (m, 1H, ArH); MALDI-MS (negative mode, matrix: ATT) m/z = 544 $[M-3Na+2H]^-$; 609.3 for $C_{20}H_{20}N_3Na_3O_{11}P_2$. (*l*)-**3k**: HPLC (prep. RP-18, 0–10 mn isocratic 2% CH_3CN, 10–60 mn linear gradient: 1–15% CH_3CN, 15 mL/mn flow): $t_R = 29.0$ mn; ^1H NMR (250 MHz, D_2O): δ 3.40–3.78 (2m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 5.33 (d, $^3J(1',2') = 3.0$ Hz, 1H, 1′-H), 5.39 (d, $^3J(5,6) = 7.8$ Hz, 1H, 5-H), 5.90 (dd, $^2J(1'',P) = 12.8$ Hz, $^3J(1'',P') = 11.3$ Hz, 1H, 1″-H), 7.15 (d, $^3J(6,5) = 7.8$ Hz, 1H, 6-H), 7.26–7.38 (m, 3H, ArH), 7.59–7.68 (m, 3H, ArH), 7.98–8.04 (m, 1H, ArH); MALDI-MS (negative mode, matrix: ATT) m/z = 544 $[M-3Na+2H]^-$; 609.3 for $C_{20}H_{20}N_3Na_3O_{11}P_2$.

Trisodium cytidin-5′-yl-[phosphonato-(quinolin-2-yl)methyl]-phosphate (**3l**)

From diallyl α-hydroxyphosphonate **14l** according to protocol A: deallylation of the triethylammonium salt **15l**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**3l**. The diastereomers (*h*)-**3l** and (*l*)-**3l**, were separated by HPLC, converted to their sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give 41 mg (combined mass of diastereomers), 19% yield from aldehyde. (*h*)-**3l**: HPLC (prep. RP-18, 0–40 mn linear gradient: 5–11.5% CH_3CN, 16 mL/mn flow): $t_R = 9.8$ min; ^1H NMR (600 MHz, D_2O): δ 3.42 (dd, $^3J(1',2') = 2.3$ Hz, $^3J(2',3') = 5$ Hz, 1H, 2′-H), 3.54 (dd, $^3J(2',3') = 5$ Hz, $^3J(3',4') = 7.2$ Hz, 1H, 3′-H), 3.78 (bd, $^3J(3',4') = 7.2$ Hz, 1H, 4′-H), 3.84 (bd, $^2J(5'a,5'b) = 11.6$ Hz, 1H, 5′a-H), 3.95 (bd, $^2J(5'a,5'b) = 11.6$ Hz, 1H, 5′b-H), 5.21-5.23 (m, 1H, 1″-H), 5.21 (d, $^3J(1',2') = 2.3$ Hz, 1H, 1′-H), 5.65 (d, $^3J(5,6) = 7.5$ Hz, 1H, 5-H), 7.04 (d, $^3J(5,6) = 7.5$ Hz, 1H, 6-H), 7.41 (dd, $^3J(5''',6''') = 7.6$ Hz, $^3J(6''',7''') = 7.5$ Hz, 1H, 6‴-H), 7.57 (dd, $^3J(6''',7''') = 7.5$ Hz, $^3J(7''',8''') = 7.8$ Hz, 1H, 7‴-H), 7.70–7.75 (m, 3H, 3‴-H, 5‴-H, 8‴-H), 8.10 (d, $^3J(3''',4''') = 8.6$ Hz, 1H, 4‴-H); ^{13}C NMR (150.9 MHz, D_2O): δ 62.1 (C5′), 66.7 (C3′), 73.8 (C2′), 78.4 ($^1J(C,P) = 150$ Hz, C1″), 80.8 (C4′), 88.8 (C1′), 95.6 (C5), 120.4 (C3′), 126.0 (C6‴), 126.3 (C8‴), 126.9 (C4a‴), 127.2 (C5‴), 129.4 (C7‴), 137.1 (C4‴), 139.9 (C6), 145.0 (C8a‴), 156.4 (C4), 159.2 (C2‴), 165.2 (C2); ^{31}P NMR (243 MHz, D_2O): δ 2.26 (bs, PO_4), 12.23 (bs, PO_3); MALDI-MS (negative mode, matrix: ATT) m/z = 589 ($[M-Na+2H]^-$, 100%), 565 ($[M-2Na+H]^-$, 30), 543 ($[M-3Na+2H]^-$, 25); 610.3 for $C_{19}H_{19}N_4Na_3O_{11}P_2$. (*l*)-**3l**: HPLC (prep. RP-18, 0-40min linear gradient: 5–11.5% CH_3CN, 16 mL/mn flow): $t_R = 10.5$ min; ^1H NMR (600 MHz, D_2O): δ 3.46 (dd, $^3J(2',3') = 5$ Hz, $^3J(3',4') = 5.6$ Hz, 1H, 3′-H), 3.60 (dd, $^3J(1',2') = 3.7$ Hz, $^3J(2',3') = 5$ Hz, 1H, 2′-H), 3.73 (bd, $^2J(5'a,5'b) = 11.8$ Hz, 1H, 5′a-H), 3.82 (bd, 1H, 4′-H), 3.94 (bd, $^2J(5'a,5'b) = 11.8$ Hz, 1H, 5′b-H), 5.34 (dd, $^3J(1'',P) = 13.2$ Hz, $^2J(1'',P) = 10.2$ Hz, 1H, 1″-H), 5.40 (d, $^3J(1',2') = 3.7$ Hz, 1H, 1′-H), 5.66 (d, $^3J(5,6) = 7.5$ Hz, 1H, 5-H), 7.26 (d, $^3J(5,6) = 7.5$ Hz, 1H, 6-H), 7.48 (dd, $^3J(5''',6''') = 7.8$ Hz, $^3J(6''',7''') = 7.5$ Hz, 1H, 6‴-H), 7.64-7.71 (m, 2H, 3‴-H, 7‴-H), 7.80 (d, $^3J(5''',6''') = 7.8$ Hz, 1H, 5‴-H), 7.85 (d, $^3J(7''',8''') = 8.1$ Hz, 1H, 8‴-H), 8.26 (d, $^3J(3''',4''') = 8.6$ Hz, 1H, 4‴-H); ^{13}C NMR (150.9 MHz, D_2O): δ 63.9 (C5′), 68.1 (C3′), 73.7 (C2′), 77.1 ($^1J(C,P) = 150$ Hz, C1″), 81.6 (C4′), 88.8 (C1′), 95.6 (C5), 120.1 (C3′), 125.8 (C8‴), 126.6 (C6‴), 126.9 (C4a‴), 127.4 (C5‴), 130.3 (C7‴), 137.9 (C4‴), 140.0 (C6), 144.6 (C8a‴), 156.6 (C4), 157.8 (C2‴), 165.2 (C2); ^{31}P NMR (243 MHz, D_2O): δ 1.87 (d, $^3J(P,P) = 31.5$ Hz, PO_4), 12.53 (d, $^3J(P,P) = 31.5$ Hz, PO_3); MALDI-MS (negative mode, matrix: ATT) m/z = 565 ($[M-2Na+H]^-$, 50%) 543 ($[M-3Na+2H]^-$, 100); 610.3 for $C_{19}H_{19}N_4Na_3O_{11}P_2$.

Trisodium cytidin-5′-yl-[phosphonato-1-(6-pyrimidinon-4-yl)methyl]-phosphate (**3m**)

From diallyl α-hydroxyphosphonate **14m** according to protocol A: deallylation of the triethylammonium salt **15m**, followed by RP-FC (C_{18}-silica gel: H_2O/EtOH 3:1) purification, and *O*/*N*-deacetylation gave (S/*R*)-**3m**, which was further purified by HPLC, converted to the sodium salt form by ion-exchange (IR 120 Na^+), precipitated from EtOH and lyophilised from water to give (S/*R*)-**3m** (18 mg, 20% yield from aldehyde). ^1H NMR (250 MHz, D_2O): δ 3.82–4.15 (m, 5H, 2′-H, 3′-H, 4′-H, 5′a,b-H), 5.37 (m, 1H, 1″-H), 5.69 (d, $^3J(1',2') = 4.0$ Hz, 0.5H, 1′-H), 5.73 (d, $^3J(1',2') = 4.8$ Hz, 0.5H, 1′-H), 6.01 (2d, $^3J(5,6) = 7.8$ Hz, 1H, 5-H), 6.41–6.50 (m, 1H, 5‴-H), 7.75 (d, $^3J(6,5) = 7.8$ Hz, 0.5H, 6-H), 7.86 (d, $^3J(6,5) = 7.8$ Hz, 0.5H, 6-H), 8.02-8.11 (m, 1H, 2‴-H); MALDI-MS (negative mode, matrix: ATT) m/z = 510 $[M-3Na+2H]^-$, 415 [M-pyr.-3Na+2H]$^-$; 577.3 for $C_{14}H_{16}N_5Na_3O_{12}P_2$.

Asymmetric synthesis of arylmethyl α(2-6)sialyltransferase inhibitors **3a–c**

Condensation of chiral α-hydroxybenzylphosphonates [21] (*S*)-**14a**,**b**,**n** and (*R*)-**14a**,**b**,**n** with cytidinephosphitamide **10** was performed according to protocol A, as described above.

Triethylammonium (N-acetyl-2′,3′-di-O-acetylcytidin-5′-yl)-[(S)-(diallylphosphonato)-phenylmethyl]-phosphate ((S)-**15a**)

From (*S*)-**14a** (25.0 mg, 93.0 μmol) according to protocol A: purification by FC (silica gel, 3:1 EtOAc-MeOH + 1% Et_3N, $R_f = 0.3$) gave (*S*)-**15a** (36.2 mg, 49% yield, $[\alpha]_D = +2$ [*c*. 1.1 EtOH]) as a colourless lyophilisate from dioxane. ^1H NMR (D_4-MeOH, 250 MHz): δ 1.29 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.05 (s, 3H, $C(O)CH_3$), 2.08 (s, 3H, $C(O)CH_3$), 2.19 (s, 3H, $NH(O)CH_3$), 3.18 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.77 (dm, 1H, $^2J(5a',5b') = 12.0$ Hz, 5a′-H), 4.09 (dm, 1H, $^2J(5b',5a') = 12.0$ Hz, 5b′-H), 4.20 (m, 1H, 4′-H), 4.45–4.63 (m, 4H, $CH_2CH=CH_2$), 5.10–5.38 (m, 6H, 2′-H, 3′-H, CH_2CH

$= CH_2$), 5.59 (dd, $^2J(1'',P) = 13.7$, $^3J(1'',P) = 11.4$ Hz, 1H, 1″-H), 5.81–5.98 (m, 2H, $CH_2CH = CH_2$), 6.14 (d, $^3J(1,2) = 5.5$ Hz, 1′-H), 7.35–7.40 (m, 3H, *o*-, *p*-ArH), 7.49 (d, $^3J(5,6) = 7.6$ Hz, 5-H), 7.55–7.58 (m, 2H, *m*-ArH), 8.32 (d, $^3J(6,5) = 7.6$ Hz, 6-H).

Triethylammonium (N-acetyl-2′,3′-di-O-acetylcytidin-5′-yl)-[(R)-(diallylphosphonato)-phenylmethyl]-phosphate ((R)-**15a**)

From (*R*)-**14a** (373 mg, 1.39 mmol) according to protocol A: purification by FC (silica gel, 3:1 EtOAc-MeOH + 1% Et_3N, $R_f = 0.3$) gave (*R*)-**15a** (918 mg, 83% yield, $[\alpha]_D = +40$ [*c*. 2.2 MeOH]) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.29 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.05, 2.09 (s, 6H, $C(O)CH_3$), 2.19 (s, 3H, $NH(O)CH_3$), 3.17 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.98 (m, 2H, 5a′-H, 5b′-H), 4.20 (m, 1H, 4′-H), 4.45–4.61 (m, 4H, $CH_2CH = CH_2$), 5.12–5.35 (m, 6H, 2′-H, 3′-H, $CH_2CH = CH_2$), 5.61 (dd, $^2J(1'',P) = 13.7$, $^3J(1'',P) = 11.3$ Hz, 1H, 1″-H), 5.81–6.00 (m, 2H, $CH_2CH = CH_2$), 6.09 (d, $^3J(1,2) = 5.4$ Hz, 1′-H), 7.25–7.38 (m, 3H, *o*-, *p*-ArH), 7.44 (d, $^3J(5,6) = 7.6$ Hz, 5-H), 7.50–7.57 (m, 2H, *m*-ArH), 8.19 (d, $^3J(6,5) = 7.6$ Hz, 6-H); ^{13}C NMR (D_4-MeOH, 63 MHz): δ 9.2 ($N(CH_2CH_3)_3$), 20.3, 20.5 ($C(O)CH_3$), 24.6 ($NHC(O)CH_3$), 47.8 ($N(CH_2CH_3)_3$), 65.5 ($J(C,P) = 3.8$ Hz, C3′), 68.9 ($J(C,P) = 5.6$ Hz, $CH_2CH = CH_2$), 69.2 ($J(C,P) = 5.3$ Hz, $CH_2CH = CH_2$), 72.3, 75.3 (C2′, C5′), 74.9 ($^1J(C,P) = 136.5$ Hz, C1″), 83.2 ($J(C,P) = 7.0$ Hz, C4′), 89.3 (C1′), 99.9 (C5), 118.4 ($CH_2CH = CH_2$), 129.4, 129.7 (ArCH),[2] 134.1 ($J(C,P) = 5.2$ Hz, $CH_2CH = CH_2$), 136.5 (ArC), 146.4 (C6), 158.0 (C2), 164.5 (C4), 171.0, 171.3, 172.9 ($C(O)CH_3$, $NHC(O)CH_3$); ^{31}P NMR (D_4-MeOH, 162 MHz): δ 0.59 (d, $^3J(P,P) = 36.6$ Hz, $P(O)O_3$), 20.16 (d, $^3J(P,P) = 36.6$ Hz, $P(O)O_2$); MALDI-MS (negative mode, matrix: ATT) m/z = 698 ($[M\text{-}HNEt_3]^-$, 100%), 658 ($[M\text{-}NEt_3\text{-}All]^-$, 38%); 800.7 for $C_{34}H_{50}N_4O_{14}P_2$.

Triethylammonium (N-acetyl-2′,3′-di-O-acetylcytidin-5′-yl)-[(S)-(diallylphosphonato)-(3-phenoxy)phenylmethyl]-phosphate ((S)-**15b**)

From (*S*)-**14b** (158 mg, 437 μmol) according to protocol A: purification by FC (silica gel, 3:1 EtOAc-MeOH + 1% Et_3N, $R_f = 0.3$) gave (*S*)-**15b** (309 mg, 79% yield, $[\alpha]_D = +1$ [*c*. 1.3 MeOH]) as a colourless lyophilisate from dioxane. ^{1}H NMR (D_4-MeOH, 600 MHz): δ 1.28 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.03, 2.07 (s, 6H, $C(O)CH_3$), 2.16 (s, 3H, $NH(O)CH_3$), 3.16 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.84 (m, 1H, 5a′-H), 4.07 (m, 1H, 5b′-H), 4.21 (m, 1H, 4′-H), 4.51–4.58 (m, 4H, $CH_2CH = CH_2$), 5.15–5.35 (m, 6H, 2′-H, 3′-H, $CH_2CH{=}CH_2$), 5.58 (m, 2H, CHP), 5.89 (m, 4H, $CH_2CH{=}CH_2$), 6.14 (d, $^3J(1,2) = 5.4$ Hz, 1′-H), 6.92–7.32 (m, 9H, ArH), 7.46 (d, $^3J(5,6) = 7.5$ Hz, 5-H), 8.31 (d, $^3J(6,5) = 7.5$ Hz, 6-H); ^{13}C NMR (D_4-MeOH, 150 MHz): δ 9.2 ($N(CH_2CH_3)_3$), 20.3, 20.5 ($C(O)CH_3$), 24.6 ($NHC(O)CH_3$), 47.8 ($N(CH_2CH_3)_3$), 65.6 (C5′), 69.0, 69.3 ($CH_2CH = CH_2$), 72.3 (C3′), 74.6 ($^1J(C,P) = 164$ Hz, CHP), 75.4 (C2′), 83.2 (d, $J(C,P) = 8.9$ Hz, C4′), 89.3 (C1′), 99.0 (C5), 118.4 ($J(C,P) = 5.4$ Hz, $CH_2CH = CH_2$), 119.1, 119.4, 120.7, 124.0, 124.6, 130.8, 130.9, 134.2 (ArCH,[2] $CH_2CH = CH_2$), 138.5 (ArC), 146.4 (C6), 158.0, 158.3, 158.8 (C2, ArCO), 164.5 (C4), 170.9, 171.1, 172.8 ($C(O)CH_3$, $NHC(O)CH_3$); ^{31}P NMR (D_4-MeOH, 162 MHz): δ 1.42 (d, $^3J(P,P) = 21.8$ Hz, $P(O)O_3$), 21.76 (d, $^3J(P,P) = 21.8$ Hz, $P(O)O_2$); MALDI-MS (negative mode, matrix: THAP) m/z = 790 ($M\text{-}HNEt_3)^-$, 100%); MALDI-MS (positive mode, matrix: DHB) m/z = 814 ($M\text{-}NEt_3 + Na)^+$, 100%); 892.8 for $C_{40}H_{54}N_4O_{15}P_2$.

Triethylammonium (N-acetyl-2′,3′-di-O-acetylcytidin-5′-yl)-[(R)-(diallylphosphonato)-(3-phenoxy) phenylmethyl]-phosphate ((R)-**15b**)

From (*R*)-**14b** (95.3 mg, 0.26 mmol) according to protocol A: purification by FC (silica gel, 3:1 EtOAc-MeOH + 1% Et_3N, $R_f = 0.3$) gave (*R*)-**15b** (167 mg, 72% yield, $[\alpha]_D = +48$ [*c*. 1.1 MeOH]) as a colourless lyophilisate from dioxane (however, *N*-deacetylation occurred prior to NMR acquisition). ^{1}H NMR (D_4-MeOH, 600 MHz): δ 1.30 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.03, 2.09 (s, 6H, $C(O)CH_3$), 3.18 (q, $^3J = 7.3$ Hz, 6H, $N(CH_2CH_3)_3$), 3.95 (m, 2H, 5′-H), 4.15 (m, 1H, 4′-H), 4.56–4.59 (m, 4H, $CH_2CH = CH_2$), 5.17–5.34 (m, 6H, 2′-H, 3′-H, $CH_2CH = CH_2$), 5.56 (m, 2H, 1″-H), 5.88 (m, 4H, $CH_2CH = CH_2$), 5.94 (d, $^3J(5,6) = 7.5$ Hz, 5-H), 6.13 (d, $^3J(1,2) = 5.8$ Hz, 1′-H), 6.97–7.33 (m, 9H, ArH), 7.84 (d, $^3J(6,5) = 7.5$ Hz, 6-H); ^{13}C NMR (D_4-MeOH, 150 MHz): δ 9.2 ($N(CH_2CH_3)_3$), 20.3, 20.5 ($C(O)CH_3$), 47.8 ($N(CH_2CH_3)_3$), 65.6 (C5′), 68.9, 69.4 ($CH_2CH = CH_2$), 72.2 (C3′), 74.5 ($^1J(C,P) = 177$ Hz, C1″), 74.9 (C2′), 82.6 ($J(C,P) = 9.3$ Hz, C4′), 88.1 (C1′), 97.2 (C5), 118.4 ($CH_2CH = CH_2$), 119.4, 119.9, 120.1, 124.1, 124.6, 130.7, 130.9, 134.2 (ArCH, $CH_2CH = CH_2$), 138.5 (ArC), 142.7 (C6), 158.3, 158.4, 158.7 (C2, ArCO), 167.5 (C4), 171.0, 171.3 ($C(O)CH_3$); ^{31}P NMR (D_4-MeOH, 162 MHz): δ 1.44 (d, $^3J(P,P) = 21.8$ Hz, $P(O)O_3$), 21.79 (d, $^3J(P,P) = 21.8$ Hz, $P(O)O_2$); MALDI-MS (negative mode, matrix: THAP) m/z = 790 ($M\text{-}HNEt_3)^-$, 100%); MALDI-MS (positive mode, matrix: DHB) m/z = 814 ($[M\text{-}NEt_3 + Na]^+$, 100%); 892.8 for $C_{40}H_{54}N_4O_{15}P_2$.

Triethylammonium (N-acetyl-2′,3′-di-O-acetylcytidin-5′-yl)-[(S)-(diallylphosphonato)-(3-trifluoromethyl)phenylmethyl]-phosphate ((S)-**15n**)

From (*S*)-**14n** (79.0 mg, 0.24 mmol) according to protocol A: purification by FC (silica gel, 3:1 EtOAc-MeOH + 1% Et_3N, $R_f = 0.3$) gave (*S*)-**15n** (187 mg, 90% yield, $[\alpha]_D = +2$ [*c*. 0.7 MeOH]) as a colourless lyophilisate from dioxane.^{1}H NMR (D_4-MeOH, 250 MHz): δ 1.27 (t, $^3J = 7.3$ Hz, 9H, $N(CH_2CH_3)_3$), 2.04, 2.06 (s, 6H, $C(O)CH_3$), 2.16 (s, 3H,

NH(O)CH_3), 3.16 (q, ^3J = 7.3 Hz, 6H, N(CH_2CH$_3$)$_3$), 3.86 (m, 1H, 5a′-H), 4.20 (m, 2H, 4′-H, 5b′-H), 4.57 (m, 4H, CH_2CH = CH$_2$), 5.12–5.60 (m, 6H, 2′-H, 3′-H, CH$_2$CH = CH_2), 5.72–6.00 (m, 3H, 1″-H, CH$_2$CH=CH$_2$), 6.15 (d, ^3J(1,2) = 5.4 Hz, 1′-H), 7.45–7.65 (m, 3H, 5-H, ArH), 7.78–7.90 (m, 2H, ArH), 8.31 (d, ^3J(6,5) = 7.6 Hz, 6-H); ^{13}C NMR (D$_4$-MeOH, 63 MHz): δ 9.2 (N(CH$_2$CH_3)$_3$), 20.3, 20.4 (C(O)CH_3), 24.7 (NHC(O)CH_3), 47.7 (N(CH_2CH$_3$)$_3$), 65.8 (J(C,P) = 4.9 Hz, C3′), 69.2 (J(C,P) = 6.9 Hz, CH_2CH=CH$_2$), 69.4 (J(C,P) = 6.7 Hz, CH_2CH = CH$_2$), 72.7 (J(C,P) = 5.7 Hz, C2′), 73.7 (^1J(C,P) = 202.8 Hz, C1″), 75.5 (C5′), 83.2 (J(C,P) = 8.8 Hz, C4′), 89.5 (C1′), 99.1 (C5), 118.6, 118.7 (CH$_2$CH = CH_2), 125.7, 126.4, 130.3, 132.8, 134.0 (ArCH, CH$_2$CH = CH$_2$), 138.0 (ArC), 146.5 (C6), 158.0 (C2), 164.5 (C4), 171.0, 171.1, 172.8 (C(O)CH$_3$, NHC(O)CH$_3$), ArCCF$_3$, CF$_3$ not detected; ^{31}P NMR (D$_4$-MeOH, 162 MHz): δ 1.82 (d, ^3J(P,P) = 27.5 Hz, P(O)O$_3$), 22.11 (d, ^3J(P,P) = 27.5 Hz, P(O)O$_2$); MALDI-MS (negative mode, matrix: ATT) m/z = 766 ([M-HNEt$_3$]$^-$, 100%), 726 ([M-NEt$_3$-All]$^-$, 24%); MALDI-MS (positive mode, matrix: DHB) m/z = 791 ([M-NEt$_3$ + Na]$^+$, 70%); 868.7 for C$_{35}$H$_{49}$F$_3$N$_4$O$_{14}$P$_2$.

Triethylammonium (N-acetyl-2′,3′-di-O-acetylcytidin-5′-yl)-[(R)-(diallylphosphonato)-(3-trifluoromethyl)phenylmethyl]-phosphate ((R)-**15n**)

From (*R*)-**14n** (53.8 mg, 0.16 mmol) according to protocol A: purification by FC (silica gel, 3:1 EtOAc-MeOH + 1% Et$_3$N, R$_f$ = 0.3) gave (*R*)-**15n** (123 mg, 88% yield, [α]$_D$ = +43 [*c*. 0.9 MeOH]) as a colourless lyophilisate from dioxane. ^{1}H NMR (D$_4$-MeOH, 250 MHz): 1.29 (t, ^3J = 7.3 Hz, 9H, N(CH$_2$CH_3)$_3$), 2.06, 2.08 (s, 6H, C(O)CH_3), 2.19 (s, 3H, NH(O)CH_3), 3.17 (q, ^3J = 7.3 Hz, 6H, N(CH_2CH$_3$)$_3$), 4.08 (m, 2H, 5a′-H, 5b′-H), 4.26 (m, 1H, 4′-H), 4.59 (m, 4H, CH_2CH = CH$_2$), 5.18–5.40 (m, 6H, 2′-H, 3′-H, CH$_2$CH = CH_2), 5.71 (dd, ^2J(1″,P) = 14.3, ^3J(1″,P) = 11.2 Hz, 1H, 1″-H), 5.82–6.01 (m, 2H, CH$_2$CH = CH$_2$), 6.10 (d, ^3J(1,2) = 3.3 Hz, 1′-H), 7.42 (d, ^3J(5,6) = 7.6 Hz, 5-H), 7.55–7.61 (m, 2H, ArH), 7.78–7.82 (m, 2H, ArH), 8.25 (d, ^3J(6,5) = 7.6 Hz, 6-H); ^{13}C NMR (D$_4$-MeOH, 63 MHz): δ 9.2 (N(CH$_2$CH_3)$_3$), 20.3, 20.5 (C(O)CH_3), 24.6 (NHC(O)CH_3), 47.8 (N(CH_2CH$_3$)$_3$), 65.8 (J(C,P) = 4.9 Hz, C3′), 69.1 (J(C,P) = 6.9 Hz, CH_2CH = CH$_2$), 69.3 (J(C,P) = 6.7 Hz, CH_2CH = CH$_2$), 72.9 (J(C,P) = 5.7 Hz, C2′), 73.6 (^1J(C,P) = 202.8 Hz, C1″), 75.6 (C5′), 83.0 (J(C,P) = 8.8 Hz, C4′), 89.6 (C1′), 98.9 (C5), 118.6 (CH$_2$CH = CH_2), 125.7, 126.3, 130.2, 132.8, 134.0 (ArCH, CH$_2$CH = CH$_2$), 138.0 (ArC), 146.3 (C6), 157.9 (C2), 164.5 (C4), 171.0, 171.2, 172.8 (C(O)CH$_3$, NHC(O)CH$_3$); ^{31}P NMR (D$_4$-MeOH, 162 MHz): δ 2.11 (d, ^3J(P,P) = 32.4 Hz, P(O)O$_3$), 22.18 (d, ^3J(P,P) = 32.4 Hz, P(O)O$_2$); MALDI-MS (negative mode, matrix: ATT) m/z = 766 ([M-HNEt$_3$]$^-$, 50%), 726 ([M-NEt$_3$-All]$^-$, 100%); MALDI-MS (positive mode, matrix: DHB) m/z = 791 ([M-NEt$_3$+Na]$^+$, 100%); 868.7 for C$_{35}$H$_{49}$F$_3$N$_4$O$_{14}$P$_2$.

Preparation of the diastereomerically pure trisodium (benzylphosphonato)cytidin-5′-ylphosphates (S)-3a–c and (R)-3a–c

Trisodium cytidin-5′-yl-[(S)-phenyl-phosphonatomethyl]-phosphate ((S)-**3a**)

From (*S*)-**15a** (34.6 mg, 43.2 μmol): deallylation, purification by RP-FC (C$_{18}$ silica gel, 3:1 H$_2$O:EtOH), *O*/*N*-deacetylation, further purification by RP-FC (C$_{18}$ silica gel, 5% ACN in 0.05 M TEAB, R$_f$ = 0.8), followed by conversion to the trisodium salt form by ion exchange using IR 120 (Na$^+$) and lyophilisation from ultra-pure water gave (*S*)-**3a** (16.9 mg, 70% yield) as a colourless lyophilisate, with spectroscopic data identical to those reported for (*h*)-**3a** [13].

Trisodium cytidin-5′-yl-[(R)-phenyl-phosphonatomethyl]-phosphate ((R)-**3a**)

From (*R*)-**15a** (376 mg, 0.47 mmol): deallylation, purification by RP-FC (C$_{18}$ silica gel, 3:1 H$_2$O:EtOH), *O*/*N*-deacetylation, further purification by RP-FC (C$_{18}$ silica gel, 5% ACN in 0.05 M TEAB, R$_f$ = 0.8), followed by conversion to the trisodium salt form by ion exchange using IR 120 (Na$^+$) and lyophilisation from ultra-pure water gave (*R*)-**3a** (194 mg, 74% yield) as a colourless lyophilisate, with spectroscopic data identical to those reported for (*l*)-**3a** [13].

Trisodium cytidin-5′-yl-[(S)-(3-phenoxy)phenyl-phosphonatomethyl]-phosphate ((S)-**3b**)

From (*S*)-**15b** (294 mg, 0.33 mmol): deallylation, purification by RP-FC (C$_{18}$ silica gel, 3:1 H$_2$O:EtOH), *O*/*N*-deacetylation, further purification by RP-FC (C$_{18}$ silica gel, 5% ACN in 0.05 M TEAB, R$_f$ = 0.8), followed by conversion to the trisodium salt form by ion exchange using IR 120 (Na$^+$) and lyophilisation from ultra-pure water gave (*S*)-**3b** (106 mg, 49% yield, [α]$_D$ = −33 [*c*. 0.1 50% aq. MeOH]) as a colourless lyophilisate, with spectroscopic data identical to those for (*h*)-**3b**.

Trisodium cytidin-5′-yl-[(R)-(3-phenoxy)phenyl-phosphonatomethyl]-phosphate ((R)-**3b**)

From (*R*)-**15b** (149 mg, 0.17 mmol): deallylation, purification by RP-FC (C$_{18}$ silica gel, 3:1 H$_2$O:EtOH), *O*/*N*-deacetylation, further purification by RP-FC (C$_{18}$ silica gel, 5% ACN in 0.05 M TEAB, R$_f$ = 0.8), followed by conversion to the trisodium salt form by ion exchange using IR 120 (Na$^+$) and lyophilisation from ultra-pure water gave (*R*)-**3b** (39.9 mg, 36% yield) as a colourless lyophilisate, with spectroscopic data identical to those for (*l*)-**3b**.

Trisodium cytidin-5′-yl-[(S)-(3-trifluoromethyl)phenyl-phosphonatomethyl]-phosphate ((S)-**3c**)

From (*S*)-**15c** (108 mg, 0.12 mmol): deallylation, purification by RP-FC (C$_{18}$ silica gel, 3:1 H$_2$O:EtOH), *O*/*N*-deacetylation,

further purification by RP-FC (C_{18} silica gel, 5% ACN in 0.05 M TEAB, $R_f = 0.8$), followed by conversion to the trisodium salt form by ion exchange using IR 120 (Na+) and lyophilisation from ultra-pure water gave (*S*)-**3c** (35.1 mg, 45% yield, $[\alpha]_D = -12$ [*c*. 0.4 50% aq. MeOH]) as a colourless lyophilisate, with spectroscopic data identical to those for (*h*)-**3c**.

Trisodium cytidin-5′-yl-[(R)-(3-trifluoromethyl)phenyl-phosphonatomethyl]-phosphate ((R)-**3c**)

From (*R*)-**15g** (62.8 mg, 72.2 μmol): deallylation, purification by RP-FC (C_{18} silica gel, 3:1 H_2O:EtOH), *O*/*N*-deacetylation, further purification by RP-FC (C_{18} silica gel, 5% ACN in 0.05 M TEAB, $R_f = 0.8$), followed by conversion to the trisodium salt form by ion exchange using IR 120 (Na^+) and lyophilisation from ultra-pure water gave (*R*)-**3c** (27.6 mg, 61% yield, $[\alpha]_D = +43$ [*c*. 0.6, 50% aq. MeOH]) as a colourless lyophilisate, with spectroscopic data identical to those for (*l*)-**3c**.

Conclusions

Inhibitors of sialyltransferases are valuable, both as important tools in the elucidation of the role of sialic acid containing glycoconjugates in biological processes, and because of their potential medicinal application in the area of cancer research and immunotherapy. Rational design of α(2-6)-sialyltransferase inhibitors based on the proposed S_N1 type mechanism has led to the development of some of the most potent sialyltransferase inhibitors described to date.

Replacement of the 2,3-dehydroneuraminic acid residue of earlier inhibitors, with planar aryl and hetaryl moieties in order to mimic the proposed planar oxocarbenium structure in the transition-state, led to the preparation of a wide range of simplified, readily accessible, yet highly potent, sialyltransferase inhibitors. Further significant features of our inhibitors include an increased distance between the anomeric carbon and the CMP leaving group and the substitution of the carboxylate group for a phosphonate moiety, producing inhibitors with two negative charges (at physiological pH) close to the glycosylation cleavage site, separated by a distance of five bonds [10–15,19,20].

A short, efficient asymmetric synthesis of the most promising benzyl inhibitors (*S*)-**3a–c** and (*R*)-**3a–c** has facilitated the assignment of the stereochemistry at the newly formed stereogenic centre and allowed comparison of the binding affinities of stereoisomeric inhibitors. Furthermore, the asymmetric route described here provides us with rapid access to large quantities of stereochemically-pure (>96% *de*) sub-micromolar inhibitors for much needed further biological investigation of this important class of transition-state analogue inhibitors.

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Supporting information available

Experimental procedures and analytic data for the following new compounds (*S*/*R*)-**12a-c**, (*S*/*R*)-**13a-c**, (*S*/*R*)-**11b**, (*S*/*R*)-**14c-m**, (*S*/*R*)-**15b-m** are available.

Notes

1. Spectroscopic data identical to those reported in Ref. [13].
2. Coincident peaks.

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