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Stereoselective Synthesis of Phosphoramidate α(2-6)Sialyltransferase Transition-State Analogue Inhibitors

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Abstract—The asymmetric synthesis of novel, potent phosphoramidate α(2-6)sialyltransferase transition-state analogue inhibitors such as (*R*)-**9** ($K_i = 68$ μM) is described, via condensation of cytidine phosphitamide **6** with key chiral, non-racemic α-aminophosphonates, prepared in >98% *ee* by Mitsunobu azidation followed by Staudinger reduction of the corresponding chiral, non-racemic α-hydroxyphosphonates.
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Sialyltransferases catalyse the addition of sialic acid residues from the β-glycoside donor cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) to the terminal position of growing oligosaccharide chains of glycoconjugate acceptors, to produce α-sialosides (Scheme 1) that influence a variety of physiologically and pathologically important processes.[1] Inhibitors of sialyltransferases are thus valuable tools in elucidating the role of sialyl residues in biological systems, in particular in light of the recent correlation between α(2-6)-sialylation of *N*-acetyllactosamine and B lymphocyte activation and immune function, which could find medicinal application.[2] As shown by us,[3–7] transition state ($TS^{\neq}$) analogues based on the recently supported[3,4,8] SN_1 type mechanism (Scheme 1) involving partial dissociation of the CMP moiety and concomitant formation of a planar oxocarbenium structure in the $TS^{\neq}$, which comprise (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 cleavage site, exhibit high affinity to sialyltransferases.[3–7,9,10]

In previous studies,[6,11] we have shown that the neuraminyl residue of $TS^{\neq}$ analogues can be replaced by a wide range of aryl and hetaryl moieties, including *m*- and *p*-substituted benzyl (Scheme 2), quinolinyl, naphthyl, and pyridyl rings, to produce readily accessible, potent aromatic inhibitors of rat liver α(2-6)-sialyltransferase. Furthermore, we have recently developed an asymmetric route to the most promising benzyl α(2-6)-ST inhibitors ([a]: R = H, OPh, CF_3, Scheme 2).[11]

To further study the influence of heteroatoms on binding affinity, we have introduced a phosphoramidate moiety[12] into our inhibitors ([b], Scheme 2). Extending our work on the development of stereoselective routes to sialyltransferase inhibitors, we report here our preliminary results on the asymmetric synthesis and bioactivity of novel, non-racemic phosphoramidate $TS^{\neq}$ analogue inhibitors, (*R*)- and (*S*)-**9**.

It was envisioned that the desired phosphoramidate inhibitors (*R*)- and (*S*)-**9** could be obtained by modifying our procedure[11] for the asymmetric synthesis of substituted benzylphosphonate derived inhibitors ([a], Scheme 2), and employing chiral, non-racemic α-aminophosphonates with standard phosphitamide coupling methodology ([b], Scheme 2).

α-Aminophosphonic acids are important isosteres of α-amino acids showing a variety of biological effects.[13] Thus several routes for the synthesis of chiral, non-racemic α-aminophosphoryl compounds have already been reported, including enzymatic[14] or chemical resolution,[15] addition of phosphite anions to *N*-glycosylnitrones,[16] and via α-azidophosphonates derived from chiral, non-racemic α-hydroxyphosphonates.[17] We

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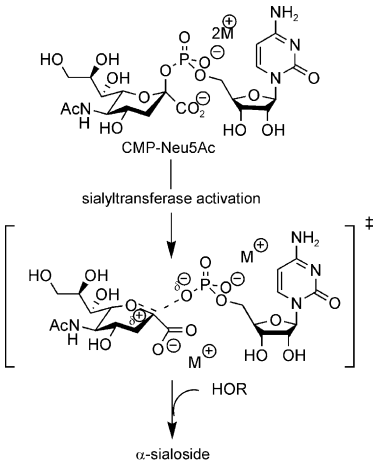
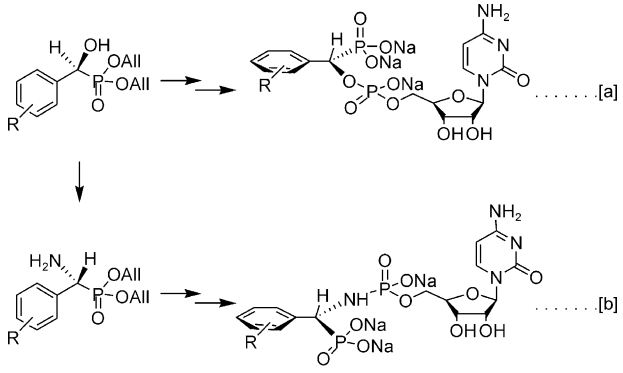


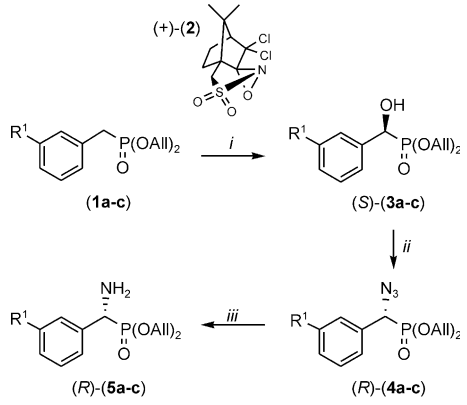
Scheme 1. Mechanism of sialyltranferase catalysed sialylation.



Scheme 2. Asymmetric routes to sialyltranferase inhibitors.

employed the latter approach for the asymmetric synthesis of α-aminophosphonates **5a–c** (Scheme 3), taking advantage of our recently reported method[18] for the synthesis of non-racemic α-hydroxyphosphonates and phosphonic acids via stereoselective hydroxylation[19] of diallyl benzylphosphonates.

The requisite chiral diallyl α-hydroxyphosphonates (*S*)-**3a–c** were prepared by reaction of the phosphoryl



Scheme 3. Reaction conditions: (i) 1.5 equiv NaHMDS, THF, −78 °C, 20 mn then 2 equiv (+)-(**2**), THF, −78 °C, 3 h, **3a–c**: 36%, 46%, 42%; (ii) PPh_3, DEAD, HN_3, 0 °C–rt, 20 h, **4a–c**: 89, 88, 98%; (iii) PPh_3, benzene, rt, 2 h, then H_2O, 50–55 °C, 5 h, **5a–c**: 85, 84, 75%.

stabilised anions of the benzylphosphonates **1a–c**, with camphorsulfonyloxaziridine (+)-**2** as described previously (Scheme 3).[18] Mitsunobu inversion reaction of the α-hydroxyphosphonates (*S*)-**3a–c** furnished the corresponding α-azidophosphonates (*R*)-**4a–c**.[20] The azides (*R*)-**4a–c** were subjected to a Staudinger reaction[21] and converted to the corresponding iminophosphoranes and subsequently hydrolysed with water in situ, to give the desired α-aminophosphonates (*R*)-**5a–c** in high yield and excellent enantiomeric excess (*ee*) (Scheme 3).[22] The enantiomers (*S*)-**5a–c** (not shown) were prepared in the analogous manner employing (−)-**2**.

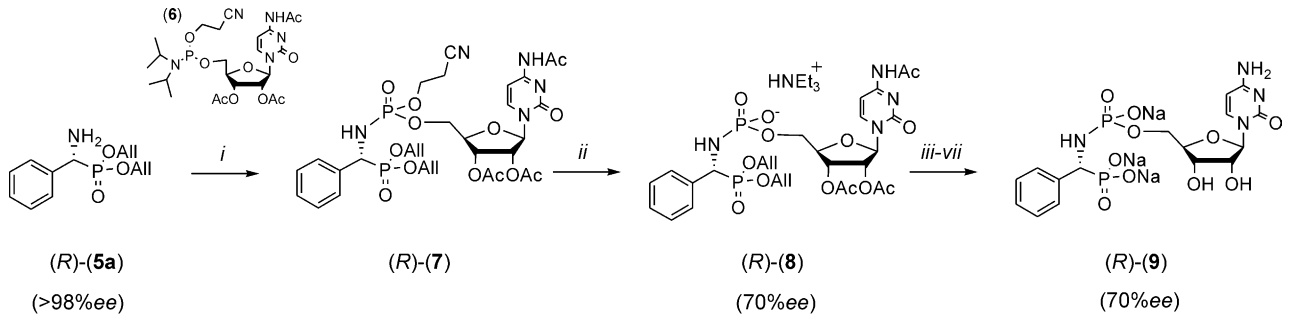
The enantiomeric purity and absolute configuration of the α-aminophosphonates (*R*)- and (*S*)-**5a–c** were established by the chemical shift differences of the ^{31}P and ^{1}H NMR spectral resonances of the corresponding amides derived from (*S*)-methyl mandelic acid (MMA) as described by Trost et al.[23] Employing the accepted model for the conformation of the (*S*)-*N*-MMA amides of α-aminophosphonates,[23] the amides derived from (*S*)-α-aminophosphonates have the phosphorus atom shielded by the mandelate phenyl ring and are thus shifted upfield relative to the (*R*)-amides.[24] Thus, for the observed chemical shift differences obtained the phosphorus atom of the (*S*)-*N*-MMA amides of (*S*)- and (*R*)-**5a–c** (Table 1), confirmed that the Mitsunobu reaction proceeded stereoselectively with inversion of configuration at the chiral centre.

Employing the conditions described by Abraham and Wagner for the synthesis of phosphoramidate amino acid diesters of antiviral nucleosides,[25] cytidine phosphitamide **6** was converted to its methyl phosphite by treatment with methanol and tetrazole, and then condensed with chiral α-aminophosphonate (*R*)-**5a** in the presence of iodine, to give, after work up and flash chromatographic purification, (*R*)-**7** in 42% yield (Scheme 4).

In our previous syntheses[5–7,10] of sialyltransferase inhibitors (e.g., [a], Scheme 2) we have employed triethylamine for the base-catalysed cleavage of the cyanoethyl phosphate protecting group, however, in the case of the phosphoramidate (*R*)-**7**, this led to degradation. This problem was solved by employing TBAF/THF for the phosphoramidate deprotection,[26] furnishing the triethylammonium salt (*R*)-**8**,[27] in good yield and high *ee*, although some racemisation was observed. Elaboration to the target compound (*R*)-**9**[28] was performed using our standard procedure,[5–7,10] comprising Pd-catalysed deallylation, followed by deacetylation, purification by reverse-phase flash chromatography, and conversion to

Table 1. NMR shifts of the (*S*)-*N*-MMA amides of (*S*)-**5a–c** and (*R*)-**5a**

Compd	$[\alpha]_D$	^{31}P NMR δ (major)	^{31}P NMR δ (minor)	Δ (ppm)	% *ee*
(*S*)-**5a**	−11	22.73 (>99)	23.04 (<1)	0.31	>98
(*S*)-**5b**	−9	22.15 (>99)	22.48 (<1)	0.33	>98
(*S*)-**5c**	−17	21.86 (96)	22.17 (4)	0.31	92
(*R*)-**5a**	+11	23.04 (>99)	22.73 (<1)	0.31	>98



(*R*)-(**5a**) (>98%*ee*) (*R*)-(**7**) (*R*)-(**8**) (70%*ee*) (*R*)-(**9**) (70%*ee*)

Scheme 4. Reagents and conditions: (i) 1 equiv cytidine-phosphitamide **6**, 4 equiv tetrazole, 2 equiv MeOH, CH_3CN, 2 h then 4.05 equiv (*R*)-(**5a**), 1 equiv I_2, THF, 3 h, 42%; (ii) 2 equiv TBAF, THF, 1 h, 40%; (iii) 20 mol.% $Pd(Ph_3)_4$, 10 equiv dimedone, THF, 18 h; (iv) RP-FC (1:3 EtOH–H_2O); (v) 25% NH_3–H_2O, 18 h; (vi) RP-FC (95:5 H_2O–CH_3CN); (vii) IR 120 Na^+ (61% over five steps).

the trisodium salt through ion-exchange chromatography (IR-120 Na^+).

The competitive inhibition of α(2-6)-ST by the phosphoramidates was investigated employing a previously reported assay[3] and gave (*R*)-**9**: $K_i = 68 \pm 24$ μM, and (*S*)-**9**: $K_i = 140 \pm 30$ μM. Phosphoramidate (*R*)-**9** binds with similar affinity to α(2-6)-ST from rat liver (E.C. 2.4.99.1) as the natural substrate CMP-Neu5Ac ($K_M = 46 \pm 7$ μM).[3] However, the analogous (*R*)-phosphodiester ([a], R = H, Scheme 2) previously prepared by us,[6] differing only by the nature of the linkage (phosphodiester vs phosphoramidate), shows approximately two orders of magnitude higher affinity. Thus, it appears that the phosphate ester linkage may be of importance in binding to α(2-6)-ST.[12,29] Furthermore, in the case of phosphodiester inhibitors, replacement of the carboxylate group by a phosphonate group, generally improves binding affinity to α(2-6)-ST by around one order of magnitude.[6] This may also contribute to the improved binding affinities seen for our *N*-(α-phosphoryl)phosphoramidate inhibitors (*R*)- and (*S*)-**9** compared to similar *N*-(α-carboxy)phosphoramidate inhibitors reported recently,[12] however, comparison of a greater number of inhibitors will be required before a clear trend emerges.

In conclusion, we have described the stereoselective synthesis of novel phosphoramidate α(2-6)-ST inhibitors such as (*R*)-**9**, via condensation of cytidine phosphitamide with key chiral, non-racemic α-aminophosphonates obtained in >98% *ee* by Mitsunobu azidation followed by Staudinger reduction of the corresponding non-racemic α-hydroxyphosphonates, providing further examples of $TS^{\neq}$ analogues exhibiting high affinity to sialyltransferases. This asymmetric route provides the first access to novel *N*-(α-phosphoryl)-phosphoramidate nucleosides, which may prove valuable in the area of phosphoramidate nucleoside prodrugs,[25] as well as useful alternatives to the difficult construction of the *N*-acyl phosphoramidate linkage found in several nucleotide antibiotics.[26]

Acknowledgements

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20. Selected data for **4a**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 4.30–4.57 (m, 4H, H1′), 4.75 (d, $^{2}J_{(H,P)}$ = 16.6 Hz, 1H, CHP), 5.15–5.33 (m, 4H, H3′), 5.72–5.95 (m, 2H, H2′), 7.35–7.50 (m, 5H, ArH); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 20.0; **4b**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 4.33–4.58 (m, 4H, H1′), 4.72 (d, $^{2}J_{(H,P)}$ = 16.6 Hz, 1H, CHP), 5.18–5.35 (m, 4H, H3′), 5.75–5.95 (m, 2H, H2′), 6.92–7.38 (m, 9H, ArH); **4c**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 4.50 (m, 4H, H1′), 4.84 (d, $^{2}J_{(H,P)}$ = 16.5 Hz, 1H, CHP), 5.18–5.32 (m, 4H, H3′), 5.75–5.92 (m, 2H, H2′), 7.45–7.68 (m, 4H, ArH); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 19.1.
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22. Selected data for **5a**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.83 (brs, 2H, NH_2), 4.27 (d, $^{2}J_{(H,P)}$ = 16.3 Hz, 1H, CHP), 4.28–4.49 (m, 4H, H1′), 5.11–5.32 (m, 4H, H3′), 5.72–5.93 (m, 2H, H2′), 7.22–7.46 (m, 5H, ArH); ^{31}P NMR ($CDCl_3$, 162 MHz) δ 26.0; MALDIMS (DHB) *m*/*z* 306 ($[MK]^+$, 100%), 294 (34), 290 ($[MNa]^+$, 30), 267.3 for $C_{13}H_{18}NO_3P$; **5b**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.72 (br.s, 2H, NH_2), 4.23 (d, $^{2}J_{(H,P)}$ = 17.7 Hz, 1H, CHP), 4.38–4.52 (m, 4H, H1′), 5.12–5.30 (m, 4H, H3′), 5.72–5.93 (m, 2H, H2′), 6.88–7.35 (m, 9H, ArH); MALDIMS (DHB) *m*/*z* 398 ($[MK]^+$, 50%), 382 ($[MNa]^+$, 100), 370 (52), 360 ($[MH]^+$, 16), 358 ($[M-All+H+K]^+$, 24), 359.4 for $C_{19}H_{22}NO_4P$; **5c**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.70 (brs, 2H, NH), 4.30–4.52 (m, 4H, H1′), 4.45 (d, $^{2}J_{(H,P)}$ = 16.4 Hz, 1H, CHP, overlapping signal), 5.12–5.30 (m, 4H, H3′), 5.70–5.92 (m, 2H, H2), 7.42–7.72 (m, 4H, ArH).
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27. Selected data for (*R*)-**8**: ^{1}H NMR (MeOH-d_4, 250 MHz) δ 1.25 (t, ^{3}J = 7.3 Hz, 9H, NCH_2CH_3), 2.05 (s, 3H, OAc), 2.08, 2.09† (s, 3H, OAc), 2.19, 2.20† (s, 3H, NHAc), 3.06 (q, ^{3}J = 7.3 Hz, 6H, NCH_2CH_3), 3.65–4.50 (m, 3H, H4′, H5a′, H5b′), 4.37–4.80 (m, 5H, CH_2–CH=CH_2, CHN), 5.10–5.42 (m, 6H, H2′, H3′ and CH=CH_2), 5.72–5.87 (m, 1.8H, CH=CH_2), 5.87–6.03† (m, 0.2H, CH=CH_2), 6.08 (d, $^{3}J_{(H1',H2')}$ = 4.6 Hz, 0.9H, H1′), 6.14† (d, $^{3}J_{(H1',H2')}$ = 5.7 Hz, 0.1H, H1′), 7.24–7.32 (m, 3H, *o*- and *p*-ArH), 7.45–7.49 (m, 3H, H5′ and *m*-ArH), 8.24 (d, $^{3}J_{(H6',H5')}$ = 7.6 Hz, 0.9H, H6′), 8.36† (d, $^{3}J_{(H6',H5')}$ = 7.6 Hz, 0.1H, H6′), †signals belonging to minor (*S*)-diastereomer (15%); MALDIMS (DHB) *m*/*z* 698 ($[M-Et_3NH]^-$, 100%), 658 ($[M-Et_3N-All]^-$, 16), 799.7 for $C_{34}H_{51}N_5O_{13}P_2$.
28. Selected data for (*R*)-**9**: ^{1}H NMR (D_2O, 600 MHz) δ 3.20† (m, 0.1H, H3′), 3.39 (t, $^{3}J_{(H3',H2')} \approx {}^{3}J_{(H3',H4')}$ = 5.4 Hz, 0.9H, H3′), 3.67–3.73 (m, 3H, H2′, H5a′, H5b′), 3.87 (m, 1H, H4′), 4.12 (dd, $^{3}J_{(H,P)}$ = 9.7 Hz, $^{2}J_{(H,P)}$ = 21.9 Hz, 1H, H1′), 5.58 (d, $^{3}J_{(H1',H2')}$ = 3.5 Hz, 0.9H, H1′), 5.75 (d, $^{3}J_{(H1',H2')}$ = 5.8 Hz, 0.1H, H1′),† 5.93† (d, $^{3}J_{(H5,H6)}$ = 7.6 Hz, 0.1H, H5), 5.99 (d, $^{3}J_{(H5,H6)}$ = 7.6 Hz, 0.9H, H5), 7.07–7.29 (m, 5H, ArH), 7.56 (d, $^{3}J_{(H6,H5)}$ = 7.6 Hz, 0.9H, H6), 7.66† (d, $^{3}J_{(H6,H5)}$ = 7.6 Hz, 0.1H, H6) †signals belonging to minor (*S*)-diastereomer (15%); ^{13}C NMR (D_2O, 63 MHz) δ 56.4 (d, $^{1}J_{(C,P)}$ = 141.8 Hz, CHP), 63.5 (C5′), 69.7 (C3′), 75.3 (C2′), 84.0 ($^{1}J_{(C,P)}$ = 14.2 Hz, C4′), 89.8 (C1′), 97.4 (C5), 127.9, 128.9, 129.0, 129.2, 129.3 (ArCH), 141.6 (ArC), 142.7 (C6), 158.7 (C2), 167.3 (C4); ^{31}P NMR (D_2O, 162 MHz) δ 8.27 (d, $^{3}J_{(P,P)}$ = 42.1 Hz, $NHPO_3$), 18.58 (d, $^{3}J_{(P,P)}$ = 42.1 Hz, PO_3); MALDIMS (CHCA) *m*/*z* 535 ($[M-Na]^-$, 19%), 513 ($[M-2Na+H]^-$, 100), 513 ($[M-3Na+2H]^-$, 56), 558.3 for $C_{16}H_{19}N_4Na_3O_{10}P_2$.
29. Compare also the phosphoramidate (*S*)-**5d** in ref 12 (K_i = 3.8 mM), with the analogous phosphodiester (*S*)-**7** (K_i = 23 μM) in ref 6, prepared by us.