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Synthesis and Evaluation of Transition-State Analogue Inhibitors of α-1,3-Fucosyltransferase**

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Glycosidases and glycosyltransferases involved in the biosynthesis of glycoconjugates associated with intercellular recognition, metastasis, and immune response represent viable therapeutic targets.^[1-7] Of particular interest are the fucosyltransferases (FucTs), which play a key role in the biosynthesis of many important fucose-containing oligosaccharides such as sialyl Lewis x (sLe^x), a determinant in numerous cell-cell interactions, for example, in inflammation^[8,9] and tumor metastasis.^[10,11] The terminal step in the biosynthetic pathway of these fucose-containing saccharides is the transfer of L-fucose from guanosine diphosphate β -Lfucose (GDP-Fuc) to the corresponding glycoconjugate acceptors,^[12,13] which is catalyzed by α -1,3-fucosyltransferase $(\alpha$ -1,3-FucT).^[14,15] Since inhibitors of fucosyltransferase may disrupt the biosynthesis of these saccharides, they have potential medicinal applications as anti-inflammatory or antitumor agents.

In the proposed transition state of α -1,3-FucT V,^[16] the pyrophosphate chelates a divalent manganese and the fucose ring adopts a flattened half-chair conformation with substantial oxocarbenium ion character (Scheme 1). Recognition of these salient features of the transition state guided our design of potential inhibitors **1–3** for fucosyltransferase.

In the present study, analogues of fucose, which incorporate the geometry or charge of the fucose moiety in the transition state, were linked to GDP, thereby retaining the contribution of GDP to binding. The cyclohexene ring of **1** was designed to mimic the flattened half-chair conformation of the fucose moiety. In addition, the carbocyclic ring is chemically more stable than its pyranose counterpart and may be more stable in vivo.

Triazoles also resemble the flattened conformation of the fucose moiety in the transition state.^[17] Previous work based on fucose-derived triazoles lacking the negative charge normally present in the GDP-fucose substrate only weakly inhibited fucosyltransferase.^[18] Therefore, a triazole was attached to GDP providing **2**, which allowed us to exploit the electrostatic interactions of the pyrophosphate and residues in the active s9ite.

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 - Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

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Scheme 1. Proposed transition state of α -1,3-FucT V.^[16]



While the cyclohexene and triazole motifs mimic the conformation of the fucose moiety in the transition state, iminocyclitols mimic the partial positive charge developed in the transition state. Iminocyclitols are good inhibitors of glycosidases,^[19,20] but are only modest inhibitors of glycosyltransferases.^[21,22] Covalently linking the negatively charged GDP to the six-membered iminocyclitol may provide a more potent inhibitor (**3**). Reported here is the synthesis and evaluation of

compounds 1–3, which are designed to mimic the transition state of the GDP-fucose moiety of fucosyltransferase-catalyzed reactions.

Since three contiguous secondary alcohols of D-mannose and L-fucose share the same configuration, D-mannose was chosen as the starting material for the construction of 1. Synthesis began with differential protection of the hydroxy groups of α -4-methoxyphenyl D-mannopyranoside (4)^[23] (Scheme 2). The C6 primary alcohol was protected as a tertbutyldiphenylsilyl (TBDPS) ether and the remaining secondary alcohols were masked as methoxymethyl (MOM) ethers to give mannopyranoside 5. Treatment with ceric ammonium nitrate (CAN)^[24,25] revealed the anomeric hydroxy group, and subsequent Albright-Goldman oxidation^[26] afforded lactone 6. Nucleophilic addition of lithium dimethyl methylphosphonate to the carbonyl group of 6 then provided tertiary alcohol 7. Reductive ring opening with sodium borohydride to generate a diol, subsequent Swern oxidation,^[27] and intramolecular Horner-Emmons olefination^[28] yielded the enone 8. Stereoselective 1,2-reduction of 8 by Luche reduction^[29] gave exclusively the pseudo-equatorial alcohol that was converted to a methyl ether with sodium hydride and iodomethane to furnish 9. The tert-butyldimethylsilyl ether was removed with tetrabutylammonium fluoride (TBAF) to give the corresponding alcohol that was phosphorylated by using dibenzyl diisopropylphosphoramidite and subsequently oxidized with 3-chloroperoxybenzoic acid (mCPBA) to give 10.^[30] Global deprotection with trifluoroacetic acid and thiophenol^[31] gave the intermediate phosphate, which was coupled to GMP using GMP-morpholidate and 1H-tetra-



Scheme 2. Reagents and conditions: a) TBDPSCl, DMAP, pyridine, 86%; b) MOMCl, DIPEA, DMAP, CH₂Cl₂, 88%; c) CAN, CH₃CN/H₂O; d) DMSO, Ac₂O, 77%; e) CH₃P(O)(OCH₃)₂, nBuLi, THF, 82%; f) NaBH₄, THF; g) DMSO, TFAA, CH₂Cl₂, -78°C; h) NaH, diglyme, 65°C, 70% for three steps; i) NaBH₄, CeCl₃, MeOH, 95%; j) NaH, MeI, THF, 100%; k) TBAF, THF, 92%; l) (*i*Pr)₂N-P(OBn)₂, 1*H*-tetrazole, CH₂Cl₂; *m*CPBA, 77%; m) 95% aq. TFA, thiophenol, 86%; n) GMP-morpholidate, 1*H*-tetrazole, pyridine, 25%. DMAP = 4-dimethylaminopyridine, DIPEA = diisopropylethylamine, TFAA = trifluoroacetic anhydride.

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zole^[32] to provide target **1**. These named reactions were also used by Marquez and Lim^[33a] and Vasella and co-workers^[33b] for the synthesis of other carbocycles.

Beginning from the known triazole **11**,^[18] catalytic hydrogenolysis generated the hydroxy groups, which were then protected as *tert*-butyldimetylsilyl ethers to give **12** (Scheme 3). Reduction of the carboxylic ester with lithium aluminum hydride (LAH), followed by phosphorylation of the resulting hydroxy group with dibenzyl diisopropylphosphoramidite and subsequent oxidation with *m*CPBA furnished intermediate **13**.^[30] Treatment with TBAF, followed by catalytic hydrogenolysis gave phosphate **14**, which underwent 1*H*-tetrazole-catalyzed coupling with GMP–morpholidate to give target **2**.

Scheme 4 illustrates the chemoenzymatic synthesis of 3. Regiospecific epoxide opening of 15^[34] with sodium azide, followed by acetylation provided acetate 16. Resolution of the diastereomers was accomplished with Pseudomonas lipase (PSL)^[35] to give products 17a and 17b, which were separable by chromatography. Treatment of 17b with 0.1N HCl unmasked the aldehyde, which underwent an FDP aldolasecatatlyzed aldol reaction^[34] with dihydroxyacetone phosphate (DHAP)^[36] to give phosphate 18. The azido-sugar 18 was then hydrogenated in the presence of platinum oxide under a hydrogen atmosphere to give the iminocyclitol phosphate 19. Whereas reductive amination using palladium catalysts reductively cleaved the phosphate group as a side reaction, no corresponding side product was observed with platinum oxide. Coupling with GMP-morpholidate and 1H-tetrazole^[32] then provided the desired iminocyclitol 3.

The transition-state analogues were evaluated by using a fluorescence-based assay that couples the production of GDP to the consumption of NADH with pyruvate kinase (Sigma) and lactate dehydrogenase (Sigma).^[16] Compounds 1-3 exhibited good competitive inhibition of both FucT V and VI, with K_i values between 6 and 13 μ M (Table 1), although no general trend of inhibition was clearly discernable. Also included in Table 1 are published inhibition data for FucT inhibitors known in the literature. The fluorinated GDPfucose analogues (e.g. 20) are the most potent inhibitors of FucTs known to date.^[37] GDP-fucose analogue 23 is a good competitive inhibitor,^[38] but inhibition activity for C-fucopyranosyl analogue 22^[39] could not be found. Transition-state analogues such as the five-membered GDP-iminocyclitol 21^[40] show competitive inhibition against FucT V, whereas the cyclohexene 24 has an affinity constant similar to that of GDP-fucose (8-34 µm).^[38] No significant inhibition of 1-3 was observed with other commercially available glycosyltransferases such as sialyltransferases.

In summary, aspects of the transition state of GDP-fucose in fucosyltransferase-catalyzed reactions were investigated through the use of transition-state analogues. The importance of charge and conformation appear to be roughly equivalent as evident by the similar inhibition constants of transitionstate analogues 1–3. The procedures described for the synthesis of 1–3 are relatively straightforward and efficient. Of particular interest in the reductive amination of the aldolase product azido-sugar phosphate 18 provides iminocyclitol phosphate 19 for subsequent coupling to give iminocyclitol



Scheme 3. Reagents and conditions: a) H₂, Pd/C, MeOH/AcOH, 85%; b) TBSOTf, 2,6-lutidine, CH₂Cl₂, 93%; c) LAH, THF, 97%; d) (*i*Pr)₂N-P(OBn)₂, 1*H*-tetrazole, CH₂Cl₂; e) *m*CPBA, CH₂Cl₂, 94% for two steps; f) TBAF, THF; g) H₂, Pd/C, EtOH, 88% for two steps; h) GMP-morpholidate, 1*H*-tetrazole, pyridine, 39%.



Scheme 4. Reagents and conditions:a) NaN₃, NH₄Cl, EtOH/H₂O, reflux, 44%; b) Ac₂O, pyridine, 95%; c) PSL 800, pH 7.0 phosphate buffer, 51% conversion; d) 0.1N HCl, 50°C; e) DHAP, FDP-aldolase, pH 6.7, 70% for two steps; f) H₂, PtO₂, MeOH/H₂O, 60%; g) GMP-morpholidate, 1*H*-tetrazole, pyridine, 40%.

Table 1. The inhibition constants were determined for FucT V and VI at 2 Km LacNAc (70 and 10 mm, respectively), pH 7.4, and 10 mm $MnCl_2$. FucT V and VI were from CalBiochem. NA = data not available.

	FucT V [µм]	FucT VI [µм]	Ref.		FucT V [µм]	FucT VI [µм]	Ref.
1	8	6	_	21	45	NA	[40]
2	8	13	_	22	NA	NA	[39]
3	13	11	_	23	$< GDP^{[a]}$		[38]
20	4	10	[37a]	24	\sim GDP–Fuc ^[b]		[38]

[a] Inhibitory activity described as better than that of GDP (K_i for GDP = 29 mM). [b] Inhibitory activity described as comparable to that of GDP-fucose (K_m for GDP-Fuc = 8–34 mM).

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nucleotide **3**. This new chemoenzymatic strategy should find use in the synthesis of other iminocyclitol nucleotides. We believe that compounds **1–3** should be useful as general inhibitors of fucosyltransferases.

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Cyanoethynylethenes: A Class of Powerful Electron Acceptors for Molecular Scaffolding**

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Tetraethynylethene (1, TEE, Scheme 1) was introduced in 1991 as a useful building block for the synthesis of one- and two-dimensional π -conjugated scaffolds, such as poly(triacetylene) oligomers, expanded radialenes, and dehydroannulenes.^[1] Incorporation of donor and acceptor substituents resulted in interesting electronic^[1e,2] and nonlinear optical properties.^[1e,3] Furthermore, the strain-free planarity allows reversible, photochemical *cis–trans* isomerization of *cis-* and *trans-*arylated TEEs without competition from undesirable thermal isomerization.^[4]



Scheme 1. Progression from tetraethynylethene (TEE, 1) to tetracyanoethene (TCNE, 7).

Cyanoethynylethenes (2–6, CEEs) are an interesting class of hybrid derivatives combining the scaffolding power of TEE with the electronic properties of tetracyanoethene (7, TCNE), which is one of the strongest organic electron acceptors known, and has been widely used in the formation of chargetransfer complexes.^[5] So far, only derivatives of **3**, with SiMe₃ (**8b**, Scheme 2) or phenyl substituents,^[6] and arylated derivatives of **6**^[7] have been reported. Hopf and Kreutzer demonstrated that there was an enhanced reactivity of the triple bonds in derivatives of **3** and **6** towards Diels–Alder reactions^[6] and in the [2+2] cycloaddition to tetrathiafulvalene, with subsequent ring opening.^[8] A similar reaction was also observed by Hirsch and co-workers at the terminal acetylene moiety of a,ω -dicyanopolyynes.^[9]

We have now extended the family of CEEs and report herein the synthesis of **9–11 a** (Scheme 2), silylated derivatives

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