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Article

Discovery of *N*-β-L-Fucosyl Amides as High-Affinity Ligands for the *Pseudomonas aeruginosa* Lectin LecB

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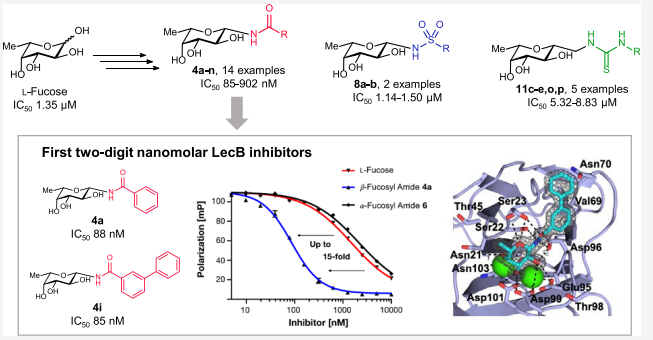
ABSTRACT: The Gram-negative pathogen *Pseudomonas aeruginosa* causes severe infections mainly in immunocompromised or cystic fibrosis patients and is able to resist antimicrobial treatments. The extracellular lectin LecB plays a key role in bacterial adhesion to the host and biofilm formation. For the inhibition of LecB, we designed and synthesized a set of fucosyl amides, sulfonamides, and thiourea derivatives. Then, we analyzed their binding to LecB in competitive and direct binding assays. We identified β-fucosyl amides as unprecedented high-affinity ligands in the two-digit nanomolar range. X-ray crystallography of one α- and one β-anomer of *N*-fucosyl amides in complex with LecB revealed the interactions responsible for the high affinity of the β-anomer at atomic level. Further, the molecules showed good stability in murine and human blood plasma and hepatic metabolism, providing a basis for future development into antibacterial drugs.



INTRODUCTION

Antimicrobial resistance is a rapidly developing threat to humanity.[1] The Gram-negative bacterium *P. aeruginosa* belongs to the problematic ESKAPE panel and is listed as the most critical drug-resistant bacterial pathogen by the WHO.[2] It is a threat to people with cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD) and to hospitalized immunocompromised patients.[3] This bacterium can form biofilms that render standard-of-care antibiotics orders of magnitude less effective.[4] Moreover, many *P. aeruginosa* strains have become multidrug resistant,[5,6] and several approaches to combat this problem are in the pipeline.[3] An alternative strategy to antibiotics are antivirulence agents or pathoblockers that instead of killing aim at disarming the bacteria to neutralize bacterial virulence and thereby provide protection to the host.[3,7]

As the major resistance mechanism, the biofilm matrix hinders penetration of antibiotics, and in addition, the embedded bacteria also reduce their metabolic activity, rendering them persistent to treatment. One promising therapeutic option is thus to interfere with biofilm formation to restore antibiotic efficacy and also to provide access to the bacteria for the immune system. For establishing the biofilm, *P. aeruginosa* expresses two extracellular lectins: LecA and LecB. They are essential constituents of the biofilm matrix,[8,9] and they establish interactions by binding to carbohydrate epitopes of the bacterial exopolysaccharides as well as to the bacterial and the host glycocalyx.[10−12] Both proteins are also crucial for initial cell adhesion as both lectins are secreted and present in the extracellular space and on the bacterial surface, where they are bound in a carbohydrate-dependent way.[9,12] The sequences of LecA and LecB have been analyzed for various clinical and environmental isolates, demonstrating the lectins' functional conservation.[13,14]

The binding of LecB to the biofilm matrix exopolysaccharide Psl[11] and its requirement for mature biofilm formation were demonstrated.[9] Furthermore, LecA is involved in host cell invasion of *P. aeruginosa* by binding to the glycosphingolipid Gb3.[15] In that process, LecA induces phosphorylation of the adaptor protein CrkII that mediates signaling across the host's plasma membrane that most likely assists the membrane engulfment.[16] Infection experiments using *lecA* or *lecB* knockout strains revealed improved lung bacterial clearance in mice and better epithelial wound healing when compared to the corresponding wild-type bacteria.[12,17]

A study on cystic fibrosis patients with chronic *P. aeruginosa* infections showed that inhalation of an L-fucose/D-galactose solution reduced the amount of bacteria in sputum.[18] In another study on *P. aeruginosa* lung infected mice, it could be

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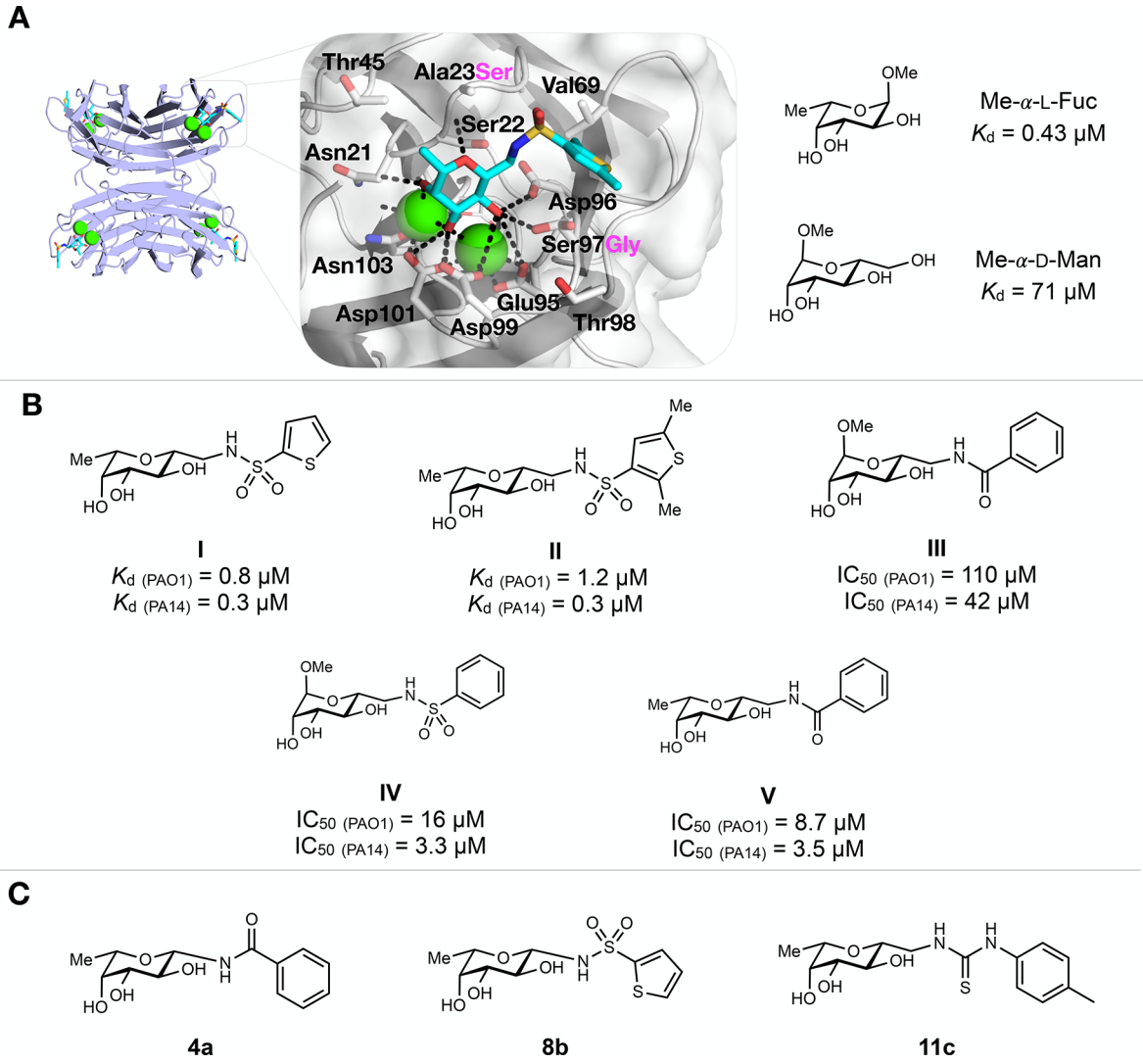

Figure 1. (A) Crystal structure of $LecB_{PA14}$ in complex with hybrid **II** (PDB: 5MAZ[40]) with the overall tetramer and a zoomed-in image of the carbohydrate binding site (green spheres: calcium ions, red spheres: waters, red: oxygens, blue: nitrogen, pink: amino acid variations in $LecB_{PAO1}$). (B) Reported glycomimetic LecB inhibitors **I**,[36] **II**, **III**–**IV**,[36,37] and **V**.[40] (C) Novel LecB inhibitors *N*-fucosyl amides (e.g., **4a**), *N*-fucosyl sulfonamides (e.g., **8b**), and fucosylmethyl thioureas (e.g., **11c**) reported in this work.

demonstrated that administration of carbohydrates in combination with antibiotics reduced the bacterial burden more efficiently than single treatments of antibiotics.[19] Therefore, the synthesis of LecA and LecB inhibitors as novel anti-infectives is an active field of research.[3,20,21]

LecA forms homotetramers and binds to D-galactosides *via* a calcium ion in its carbohydrate binding site.[22] Several monovalent galactosides were synthesized as LecA inhibitors reaching moderate binding in the micromolar range.[22−25] In contrast, divalent galactoside inhibitors with simultaneous binding to two adjacent carbohydrate binding sites of the LecA tetramer gave low nanomolar inhibitors.[20,26−28] Novel concepts were also reported such as the development of covalent lectin inhibitors,[29] addressing a subpocket between the two adjacent carbohydrate binding sites,[30] or the development of noncarbohydrate glycomimetics.[31,32]

LecB also forms homotetramers and possesses two calcium ions per carbohydrate binding site mediating binding to its fucoside or mannoside ligands.[33] The affinity of fucosides is increased compared to mannosides due to an additional lipophilic interaction of the fucose C6 methyl group with the protein at Thr45, resulting in submicromolar binding (Figure1A, K_d of Me-α-L-Fuc = 0.43 μM,[34] K_d of Me-α-D-Man = 71 μM[34]). Furthermore, it was demonstrated that the CH_2OH group of mannosides adopts a sterically hindered position when bound to LecB.[35] However, the co-crystal structure of fucose in complex with LecB[33] revealed a subpocket next to the anomeric center that was subsequently addressed in our program for LecB inhibitors (Figure 1, compounds **I**–**V**.[36−39] A small cleft between the carbohydrate binding site and said subpocket is surrounded by amino acids Ser22 and Asp96. The subpocket itself differs slightly between the two *P. aeruginosa* strains.[13]

Consequently, we have designed fucose−mannose hybrid *C*-glycoside glycomimetics[35,36,40] combining the properties of our first set of D-mannose-derived inhibitors[37] with L-fucose, which resulted in submicromolar affinities ($K_{d\ (PAO1)}$ (**I**) = 0.83 μM,[40] $K_{d\ (PA14)}$ (**I**) = 0.29 μM,[40] Figure 1B), oral bioavailability in mice, and good antibiofilm activity *in vitro*. Our initial mannosyl amides and sulfonamides as well as fucose−mannose hybrid molecules targeted this subpocket, and the interactions could be explained on the basis of several X-ray LecB

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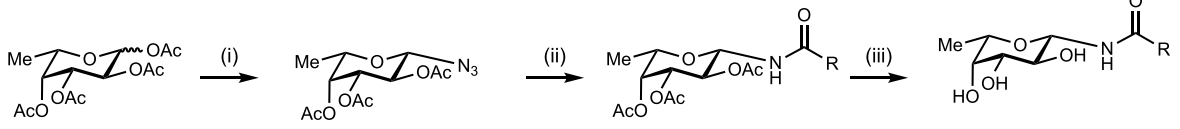
J. Med. Chem. 2022, 65, 14180−14200

Scheme 1. Synthesis of LecB Ligands β-Fucosyl Amides (4a−n), α-Fucosyl Amide (6), Fucosyl Sulfonamides (8a and b), and β-Fucosylmethyl Thioureas (11c, d, e, o, p)[a]

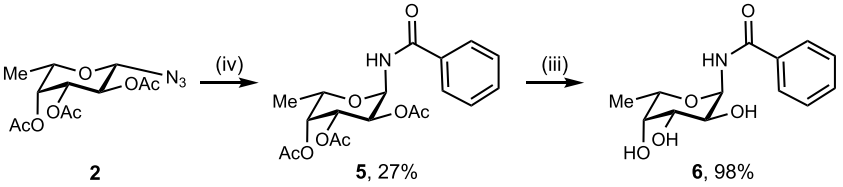
β-Fucopyranosyl Amides



1 → **2**, 77% → **3a–n** → **4a–n**

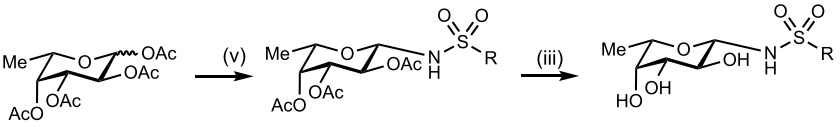
3a, 47%	**3h**, 46%	**4a**, 94%	**4h**, 47%
3b, 71%	**3i**, 27%	**4b**, 88%	**4i**, 89%
3c, 65%	**3j**, 43%	**4c**, 98%	**4j**, 84%
3d, 38%	**3k**, 12%	**4d**, 89%	**4k**, 85%
3e, 53%	**3l**, 66%	**4e**, 99%	**4l**, 99%
3f, 48%	**3m**, 33%	**4f**, 72%	**4m**, 96%
3g, 37%	**3n**, 57%	**4g**, 99%	**4n**, 85%

α-Fucopyranosyl Amide



2 → **5**, 27% → **6**, 98%

Fucopyranosyl Sulfonamides

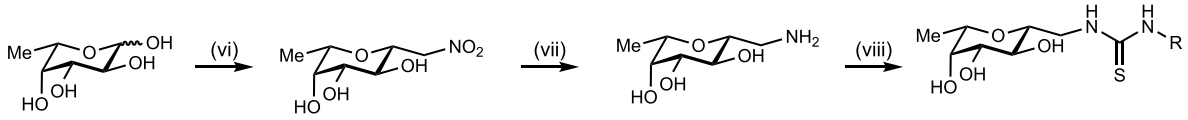


1 → **7a–b** → **8a–b**

7a, 47%
7b, 59%

8a, 75% (α/β = 33:67)
8b, 24% (α/β = 18:82)

β-Fucopyranosylmethyl Thioureas



L-Fucose → **9**, 46% → **10**, 99% → **11c–e, o,p**

11c, 71%
11d, 79%
11e, 71%
11o, 74%
11p, 80%

R=			
	a, Ph-	**i**, *m*Ph-C_6H_4-
	b, 2-Thiophenyl-	**j**, Me-
	c, *p*CH_3-C_6H_4-	**k**, $(CH_3)_2$CH-
	d, *p*MeO-C_6H_4-	**l**, (*E*)-PhCH=CH-
	e, *p*NO_2-C_6H_4-	**m**, 2-Naphthyl-
	f, *p*Cl-C_6H_4-	**n**, 2-Furanyl-
	g, *p*CF_3-C_6H_4-	**o**, *p*Et-C_6H_4-
	h, *m*CH_3-C_6H_4-	**p**, *p*F-C_6H_4-

[a]Reagents and conditions: (i) $(CH_3)_3SiN_3$, $SnCl_4$, CH_2Cl_2, 25 °C, 1.5 h; (ii) RCOCl, PPh_3, Et_3N, CH_2Cl_2, 0−25 °C, o.n.; (iii) $NaOCH_3$, MeOH, −25 to −15 °C or 0 °C, o.n. or 1.5 h; (iv) 1. PPh_3, CH_3NO_2, 4 Å molecular sieves, reflux, 24 h; 2. *S*-(pyridin-2-yl) benzothioate, $CuCl_2 \cdot H_2O$; (v) phenylsulfonamide or thiophene-2-yl-sulfonamide, $BF_3 \cdot OEt_2$, CH_3CN, 25 °C, 24 h; (vi) 1. CH_3NO_2, $NaOCH_3$ (cat.), DMSO, 25 °C, 6 h; 2. HCl (1 M, pH = 4), H_2O, reflux, o.n.; (vii) Pt/C, H_2, CH_3OH, 25 °C, 48 h; (viii) isothiocyanates, CH_3OH, 0−25 °C, o.n.

structures for both strains.[36,37,40] We demonstrated that the mannose sulfonamides inhibited LecB better than the corresponding amides ($IC_{50\ (PAO1)}$ (**III**) = 110 μM, $IC_{50\ (PA14)}$ (**III**) = 42 μM, $IC_{50\ (PAO1)}$ (**IV**) = 16 μM, $IC_{50\ (PA14)}$ (**IV**) = 3.3 μM).[36,37] Due to the different geometry in the sulfonamide moiety, these molecules can circumvent a steric clash of the amides with Ser97 on $LecB_{PA14}$.[40] However, the fucose−mannose hybrid amides were as active as the mannose sulfonamides ($IC_{50\ (PAO1)}$ (**V**) = 8.7 μM, $IC_{50\ (PA14)}$ (**V**) = 3.5 μM) due to hydrophobic interactions of their methyl groups (C6) with Thr45.[40] Shifting the amide/sulfonamide linker function further toward this subpocket by using an

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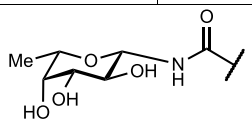
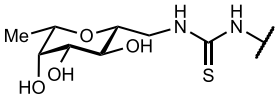
Table 1. Inhibition of $LecB_{PAO1}$ by Fucosyl Amides, Sulfonamides, and Fucosylmethyl Thioureas in a Competitive Binding Assay[23] with L-Fucose Included as a Positive Reference Compound (IC_{50} = 1350 ± 40 nM)[a]

No.	Structure	IC_{50} [nM]	No.	Structure	IC_{50} [nM]
					
4a	Ph-	88 ± 12	**4h**	*m*CH_3-C_6H_4-	120 ± 13
4b	2-Thiophenyl-	122 ± 21	**4i**	*m*Ph-C_6H_4-	85 ± 16
4c	*p*CH_3-C_6H_4-	110 ± 17	**4j**	CH_3-	902 ± 69
4d	*p*CH_3O-C_6H_4-	138 ± 20	**4k**	$(CH_3)_2CH$-	155 ± 18
4e	*p*NO_2-C_6H_4-	204 ± 44	**4l**	(*E*)-PhCH=CH-	302 ± 43
4f	*p*Cl-C_6H_4-	130 ± 19	**4m**	2-Naphthyl-	92 ± 13
4g	*p*CF_3-C_6H_4-	211 ± 41	**4n**	2-Furanyl-	272 ± 26
6	α-Fucosyl benzamide	2324 ± 432			
8a	Fucosyl benzene-sulfonamide	1496 ± 512 (α/β = 33:67)	**8b**	Fucosyl thiophene-2-sulfonamide	1144 ± 247 (α/β = 18:82)
					
11c	*p*CH_3-C_6H_4-	7387 ± 473	**11o**	*p*CH_3CH_2-C_6H_4-	8112 ± 374
11d	*p*CH_3O-C_6H_4-	7658 ± 288	**11p**	*p*F-C_6H_4-	8831 ± 103
11e	*p*NO_2-C_6H_4-	5315 ± 292			

[a]IC_{50} and std. dev. determined from three independent experiments.

elongated heptose derivative resulted in a loss in affinity for $LecB_{PAO1}$ ($IC_{50} \geq 82\ \mu M$).[38]

In the present work, we further assessed the positioning and nature of the linking unit between the carbohydrate and the pharmacophore targeting the additional subpocket in LecB. To this end, we shortened the linking function in those molecules by removing a methylene group and combined the fucose pharmacophore with amide and sulfonamide functions and, furthermore, replaced the previously reported linkers in the hybrid-type molecules with thioureas.

RESULTS AND DISCUSSION

Design of Fucosyl Amides and Sulfonamides and Fucosylmethyl Thioureas. To study the influence of the linking units between fucose and aromatic pharmacophores targeting the subpocket in LecB, we replaced the known amide and sulfonamide linkers of previous hybrid-type molecules **I** and **V** (Figure 1). First, we modified the chemical nature of the linker and introduced thioureas providing hydrogen-bond donor/acceptor properties, resulting in molecules such as **11c** (Figure 1C) with altered geometry of the linking unit and elongation. Then, we shortened the molecules into analogs devoid of the methylene group in compounds **I** and **V** and designed *N*-fucosides of amides **4** and sulfonamides **8** to assess hydrogen bond formation with the amino acids such as Ser22 (Figure S1).

These molecules were first docked *in silico* into LecB. For fucosylmethyl *p*-tolylthiourea (**11c**), the carbohydrate moiety superimposes with fucose bound to LecB (Figure S1), while the thiourea forms hydrogen bonds with the backbone of Asp96 and with its side chain. Interestingly, the software docked the thiourea function in its tautomeric thiol form, although the thione is dominating in aqueous solutions.[41] The docked binding pose of β-fucosyl benzamide (**4a**) showed again an identical orientation of the fucose, while its amide serves as a hydrogen bond acceptor for the side chain of Ser22. In addition, the aromatic ring forms lipophilic contacts with Gly24 and Val69 of the adjacent pocket (Figure S1).

Synthesis of Amides, Sulfonamides, and Thioureas. β-Fucosyl amides were obtained in a three-step synthesis from fucose tetraacetate **1** (Scheme 1). Transformation of the tetraacetate into azide **2** was achieved in good yield (77%). This azide was further converted in a Staudinger reduction followed by acylation with acyl chlorides to the corresponding protected β-fucosyl amides **3a**−**n** in yields of 12−71%. The target amides **4a**−**n** were then obtained after deacetylation under Zemplén conditions (47−99%). Furthermore, one α-fucosyl amide, benzamide **6**, was also synthesized to serve as a control molecule. **6** was obtained over two steps commencing from azide **2** by an activation with triphenylphosphine under reflux to form the α-oxazoline intermediate, which was coupled with a thiopyridyl ester of benzoic acid using Damkaci and DeShong's[42] conditions to form the protected α-fucosyl amide **5** in 27% yield. The latter compound was finally deprotected to give the α-anomer **6** in 98% yield.

Two representative examples of fucosyl sulfonamides **8a** and **8b** were synthesized by *N*-glycosylation of the respective sulfonamides. To this end, the sulfonamide acceptors were treated with tetraacetate **1** as donor under Lewis acid catalysis, and the pure β-glycosides **7a** and **7b** were obtained in good yields. Unfortunately, the subsequent deprotection step using sodium methoxide inevitably resulted in anomerization, and the test compounds were obtained as anomeric mixtures.

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Table 2. Isothermal Titration Calorimetry of LecB with Fucosyl Benzamides β-4a and α-6[a]

compound	ΔG [kJ mol^{-1}]	K_d [nM]	n	ΔH [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]
4a	−38.6	195 ± 97	1.0 ± 0.1	−29.4 ± 1.3	9.2 ± 0.4
6	−32.2	2310 ± 350	0.9 ± 0.1	−28.0 ± 1.7	4.2 ± 1.3

[a]Means and std. dev. were calculated from three independent titrations.

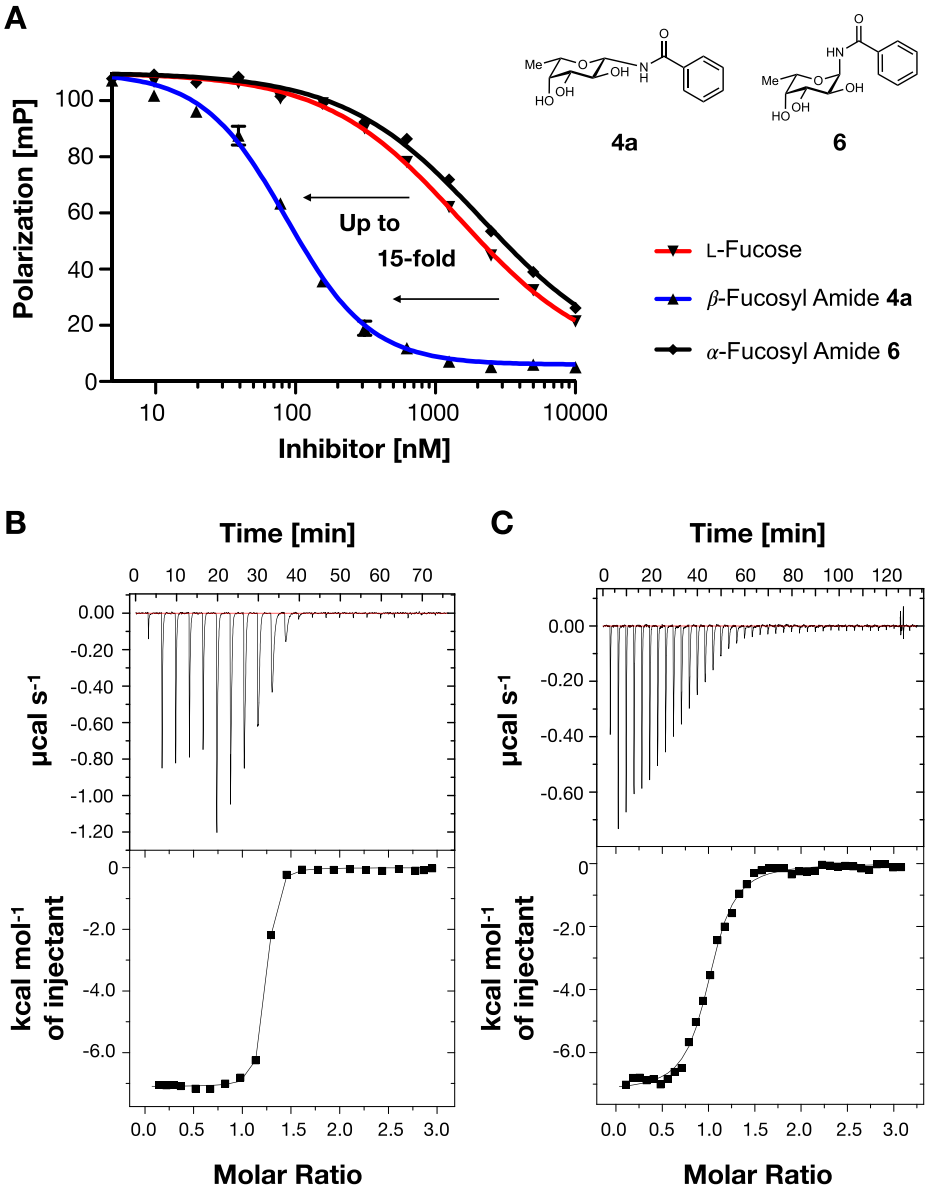

Figure 2. Biophysical analysis of fucosyl benzamides β-**4a** and α-**6** with $LecB_{PAO1}$: (A) competitive binding assay based on fluorescence polarization shows a 15-fold increase of LecB inhibition for β-**4a** compared to its α-anomer **6**; (B) isothermal titration calorimetry of β-fucosyl benzamide **4a** and of (C) α-fucosyl benzamide **6** against LecB. Both experiments show one respective titration of each fucosyl benzamide from one of the independent replicates; for A, error bars correspond to those from technical triplicates of one independent replicate.

Similar results have been reported for the synthesis of tosylated *N*-glucosides.[43−45]

The β-fucosylmethyl thioureas **11c−e**, **o**, and **p** were obtained in a three-step synthesis from L-fucose. Optimized Henry[46] reaction conditions of fucose and nitromethane were used to obtain condensation product **9**. After reduction of nitro **9** to amine **10**, the latter was reacted with various isothiocyanates to give the thioureas **11c−e**, **o**, and **p** in good yields (71−80%).

In total, 22 fucose derivatives were synthesized (Scheme 1), among which are 14 β-fucosyl amides, 1 α-fucosyl amide, 2 fucosyl sulfonamides obtained as α/β mixtures, and 5 β-fucosylmethyl thioureas.

Evaluation of LecB Binding in Biophysical Assays. All ligands were then tested in a competitive binding assay[37] based on fluorescence polarization for dose-dependent inhibition of $LecB_{PAO1}$. Due to obtained high affinities of the β-fucosyl amides, the assay was slightly modified, and a lower LecB concentration of 75 nM was used (Table 1). Therefore, the

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J. Med. Chem. 2022, 65, 14180−14200

obtained data for L-fucose were also slightly lower with an IC_{50} = 1.35 ± 0.04 μM compared to the reported value of 2.74 μM.[37]

In this assay, all tested β-fucosyl amides inhibited LecB in the nanomolar range. Acetamide **4j** showed the weakest inhibition among the series, giving an IC_{50} of 902 ± 69 nM. The affinity increased among this compound class when the acetamide was replaced with larger substituents: replacing its methyl with aromatic rings such as 2-furanoyl (IC_{50} (**4n**) = 272 ± 26 nM) or 2-thiophenyl (IC_{50} (**4b**) = 122 ± 21 nM) or a phenyl ring increased the affinity toward LecB up to 10-fold into the two-digit nanomolar range (IC_{50} (**4a**) = 88 ± 12 nM), which rendered fucosyl benzamide (**4a**) 15-fold more potent than L-fucose. Introducing electron-donating substituents on the phenyl ring (e.g., in **4c, 4d**, **4f**, and **4h**) had a negligible effect on affinity, whereas the strongly electron-withdrawing substituents in **4e** and **4g** reduced the affinity by a factor 2. Extension of the ring system into a naphthyl (IC_{50} (**4m**) = 92 ± 13 nM) or a biphenyl (IC_{50} (**4i**) = 85 ± 16 nM) residue resulted in a similarly high affinity as the benzamide **4a**. However, changing the configuration at the anomeric center from β-glycoside **4a** to its isomeric α-anomer **6** resulted in a 26-fold drop in affinity (IC_{50} (**6**) = 2324 ± 432 nM).

Some β-fucosyl amides have been reported and tested against the *N*-terminal domain of BC2L-C, a lectin from *Burkholderia cenocepacia* that also binds fucose but displays a different sequence, structure, and binding site architecture compared to LecB.[47,48] However, those compounds showed only weak BC2L-C inhibition (K_d = 0.94−7.85 mM).

The fucosyl sulfonamides **8a** and **8b** were tested as anomeric mixtures and showed an affinity comparable to L-fucose (IC_{50} (**8a**) = 1496 ± 512 nM and IC_{50} (**8b**) = 1144 ± 247 nM). Since the β-anomer was the major anomer present in both cases (67 and 82%), we assigned a reduced LecB binding potency compared to the synthesized β-fucosyl amides since 18% α-impurity in case of **8b** cannot reduce the affinity by >10-fold. When comparing these results to our previous β-fucosylmethyl sulfonamides for $LecB_{PAO1}$ (IC_{50} = 0.97−1.80 μM[36]), we conclude a comparable activity of the sulfonamides independent of the presence of the methylene group; e.g., 2-thiophene **8b** lacking the methylene group is only slightly more active (factor 1.6) than its β-fucosylmethyl homolog (IC_{50} = 1.8 μM[40]).

The β-fucosylmethyl thioureas were also tested and proved 100-fold less active than the best carboxamides reported here (IC_{50} (**11c−e**, **11o**, **11p**) = 7.3−8.8 μM). We observed a strong decrease in the affinity of the thioureas compared to the previously reported sulfonamides **I** or **II**, although they were as potent as the fucosylmethyl carboxamides **V**.

The strong decrease in affinity for LecB observed here between the very active fucosyl amides and the less active sulfonamides could possibly result from two factors. First, the increased acidity of the sulfonamides compared to the carboxamides could impact their hydrogen-bonding properties. Second, the geometry of the substituents of an amide or a sulfonamide differs from planar trans to staggered gauche, which results in an altered orientation of the linked pharmacophores.[49−51]

To validate the high affinity of the described LecB inhibitors in an orthogonal assay, we analyzed the two anomers of fucosyl benzamides, α-**6** and β-**4a**, by isothermal titration calorimetry (Table 2, Figure 2). The K_d of β-fucosyl amide **4a** obtained by ITC was 195 ± 97 nM and thus somewhat higher than the IC_{50} of 88 ± 12 nM obtained in the competitive binding assay. The binding enthalpy ΔH = −29.4 ± 1.3 kJ mol^{-1} was similar to the one reported for L-fucose (ΔH = −31.2 kJ mol^{-1}),[52] but the favorable entropic contribution $T\Delta S$ = 9.2 kJ mol^{-1} was much higher (L-Fuc = 0.3 kJ mol^{-1}),[52] explaining the increase in binding affinity. α-Fucosyl benzamide (**6**) showed a K_d of 2.3 ± 0.4 μM corresponding to the one previously reported for other types of α-fucosyl amides and LecB binding (K_d = 1.2−2.1 μM[53]). The enthalpy of LecB-binding of the α-anomer **6** (ΔH = −28.0 kJ mol^{-1}) was nearly identical to the one observed for the β-anomer, while the entropic contribution was much lower for the α-anomer ($T\Delta S$ = 4.2 kJ mol^{-1}). In general, α-linked substituents at the anomeric center of fucose point toward the solvent when bound to LecB, while β-linked substituents are oriented toward the protein surface, and thus, the observed difference in binding entropy possibly resulted from displaced protein-bound water molecules for β-benzamide **4**. Favorable entropy of binding is very unusual in protein−carbohydrate interactions but appears to be a signature of this class of two-calcium lectins[54] as rationalized by analysis of water dynamics through neutron crystallography.[55] Optimizing this favorable thermodynamic contribution through water displacement appears here as a valuable strategy. The low flexibility of the β-glycosyl amide containing ligand is also a favorable factor to avoid an entropy barrier.

X-ray Crystallography of α- and β-Fucosyl Amides in Complex with LecB. To analyze the interactions of the fucosyl amides at the atomic level, we co-crystalized compounds **4a**, **4i**, and **6** with $LecB_{PAO1}$. Co-crystals were obtained for all compounds, but **4a** in complex with LecB diffracted to a lower resolution. One data set was collected for β-benzamide **4a** in complex with LecB and was solved at 2.5 Å resolution with two tetramers in the asymmetric unit. The electron density was poor for one of the tetramers and in some binding sites and not always well defined for the aglycon, so we decided not to refine it. The data sets obtained for β-biphenyl **4i** and α-benzamide **6** in complex with LecB were of high quality, and the structures were solved at high resolutions (**4i**: 1.55 Å, **6**: 1.50 Å) (Figure 3, Table S4 for data quality). In both cases, the four carbohydrate binding sites of LecB were occupied by the ligands **4i** or **6**, and the fucose moiety of both ligands was firmly bound to the calcium ions inside the carbohydrate binding site as reported previously for the fucose/LecB complex (PDB: 1GZT[33]). In both ligands, the three hydroxy groups—OH2, OH3, and OH4—bind to the calcium ions, and their C6 methyl group interacts with Thr45 and Ser23 *via* hydrophobic contacts (Figures S2 and S3 for individual protomers).

In the complex of α-fucosyl benzamide **6** (Figure 3A, Figure S2), the amide nitrogen points away from the surface of the protein and is integrated in the hydrate layer where it loosely binds to Thr98(NH) *via* one water molecule. Its carbonyl oxygen atom points toward the binding site and forms one hydrogen bond with Ser23 (2.47−3.25 Å). Additionally, a hydrogen bond *via* water to Asp96 can be observed. The aromatic ring of the benzamide is rotated out of the plane of the amide bond. Its orientation depends on a crystal contact based on an edge-to-face interaction with another benzamide. Further, the benzamide ring forms loose lipophilic interactions with Gly97 (3.76−4.76 Å) that slightly depend on the rotation of the aromatic ring. Thus, the aromatic ring of α-fucosyl benzamide **6** only weakly contributes to the compounds' binding affinity toward LecB.

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J. Med. Chem. 2022, 65, 14180−14200

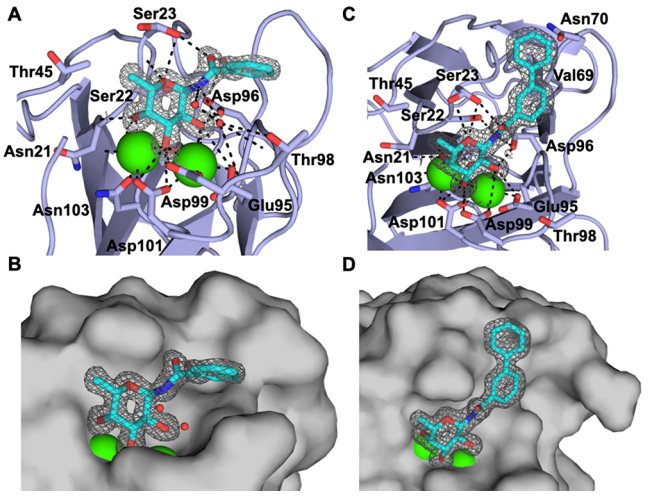
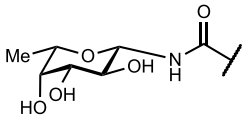


Figure 3. Co-crystal structures of (A, B; protomer B) α-fucosyl amide **6** and (C, D; protomer D) β-fucosyl amide **4i** in complex with LecB (PDB: 8AIY for **4i**, 8AIJ for **6**). Electron density is displayed at 1σ, ligands and amino acid residues in the binding site are shown as sticks, water molecules are in red, and Ca^{2+} ions are green spheres. Dashed lines indicate hydrogen-bonding interactions of the specific ligand with the protein.

In the LecB complex of β-fucosyl biphenylamide **4i** (Figure 3C, Figure S3), the amide function occupies similar orientations in protomers B−D and differs slightly in protomer A. In the latter, the amide NH of **4i** serves as a hydrogen bond donor for Ser22(OH) that makes an ion−dipole interaction with Asp96(COO^-). In protomers B−D, there is a rotation of the carbonyl oxygen by approximately 60°. This allows the carbonyl oxygen to establish a hydrogen bond with Ser23-(OH) facing toward the binding site (Figure 3C). The rotation further brings the NH in a dipole−ion interaction with Asp96, which could be an explanation for the high binding affinity of the β-fucosyl amides. The directly attached phenyl ring forms lipophilic contacts with Gly24 and Val69. The distal ring is largely solvent exposed but also forms hydrophobic contacts with Asn70. This rather small additional contact area could explain the only very small affinity increase of biphenyl **4i** compared to benzamide **4a** in the competitive binding assay.

To better understand the high affinity of fucosyl biphenylamide **4i**, we compared the two co-crystal structures of *manno*-cinnamide **S1** (PDB: 5A3O, K_d = 18.5 μM against $LecB_{PAO1}$[39]) or fucose−mannose hybrid **II** (PDB: 5MAZ, $LecB_{PA14}$, K_d = 0.8 ± 0.1 μM against $LecB_{PAO1}$[36]) with biphenyl fucosylamide **4i** in complex with $LecB_{PAO1}$ (Figure S4).

Table 3. Mouse and Human Plasma Stability and Plasma Protein Binding of α-/β-Fucosyl Amides and Sulfonamides[a]

No.	Structure	Plasma		PPB	
		Mouse $t_{1/2}$ [min]	Human $t_{1/2}$ [min]	Mouse [%]	Human [%]
					
4a	Ph-	n.d.	n.d.	38 ± 5	49 ± 16
4b	2-Thiophenyl-	stable	stable	28 ± 10	70 ± 26
4c	*p*CH_3-C_6H_4-	130	stable	n.d.	31 ± 9
4d	*p*CH_3O-C_6H_4-	stable	stable	36 ± 14	12 ± 3
4e	*p*NO_2-C_6H_4-	144	stable	46 ± 3	57 ± 16
4f	*p*Cl-C_6H_4-	24	stable	n.d.	55 ± 18
4g	*p*CF_3-C_6H_4-	stable	stable	58 ± 19	43 ± 29
4h	*m*CH_3-C_6H_4-	85	stable	45 ± 20	35 ± 14
4i	*m*Ph-C_6H_4-	119	stable	100 ± 0	90 ± 0
4j	Me	n.d.	n.d.	-	-
4k	$(CH_3)_2CH$-	stable	207	21 ± 13	12 ± 9
4l	(*E*)-PhCH=CH-	145	stable	18 ± 6	33 ± 14
4m	2-Naphthyl-	131	stable	76 ± 21	68 ± 6
4n	2-Furanoyl	n.d.	n.d.	-	-
6	α-Fucosyl benzamide	stable	stable	24 ± 8	78 ± 20
8a	Fucosyl benzene-sulfonamide	stable	stable	33 ± 7	50 ± 7
8b	Fucosyl thiophene-2-sulfonamide	123	112	100 ± 0	15 ± 9

[a]n.d. = not detected; stable ≥240 min.

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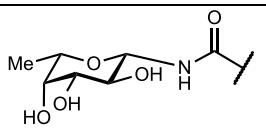
J. Med. Chem. 2022, 65, 14180−14200

Table 4. Metabolic Stability of α/β-Fucosyl Amides and Sulfonamides in Mouse or Human Liver Microsomes

No.	Structure	Mouse		Human	
		$t_{1/2}$ [min]	Cl_{int} [μL min^{-1} mg^{-1} [protein]]	$t_{1/2}$ [min]	Cl_{int} [μL min^{-1} mg^{-1} [protein]]
					
4a	Ph-	27.7	179.3	23.7	58.4
4b	2-Thiophenyl-	> 60	< 23	34.8	39.8
4c	*p*CH_3-C_6H_4-	22.4	61.8	11.5	120.4
4d	*p*MeO-C_6H_4-	> 60	< 23	161.6	8.6
4e	*p*NO_2-C_6H_4-	50.2	27.5	32.6	42.5
4f	*p*Cl-C_6H_4-	69.4	20.0	119.1	11.6
4g	*p*CF_3-C_6H_4-	161.8	8.6	229.7	6.0
4h	*m*CH_3-C_6H_4-	47.0	29.5	146.4	9.5
4i	*m*Ph-C_6H_4-	75.3	18.4	> 60	< 23
4j	CH_3-	> 60	< 23	86.4	16.0
4k	$(CH_3)_2CH$-	> 60	< 23	25.2	55.0
4l	(*E*)-PhCH=CH-	> 60	< 23	247.2	5.6
4m	2-Naphthyl-	27.6	50.2	65.7	21.1
4n	2-Furanyl-	104.4	13.3	59.6	23.2
6	α-Fucosyl benzamide	89.9	15.4	83.7	16.5
8a	α/β -Fucosyl benzenesulfonamide	> 60	< 23	> 60	< 23
8b	α/β-Fucosyl thiophene-2-sulfonamide	56.1	24.7	43.9	31.5

Superposition of the complexes $LecB_{PAO1}$-**4i** and $LecB_{PA14}$-**S1** clearly shows the effect of the additional methylene group of **S1**. The amide NH of **4i** points in between Ser22 and Asp96 to form a hydrogen bond or dipole−ion interaction. On the other hand, the amide NH of **S1** is shifted by the methylene group and points beyond the carboxylic acid of Asp96 (Figure S4), therefore being unable to form either a hydrogen bond with Ser22 or an efficient dipole−ion interaction with Asp96.

Also, for the fucose−mannose hybrid **II**, the additional CH_2 spacer prevents an interaction of the sulfonamide NH with Ser22 and Asp96 (Figure S5). But in contrast to the amides, the nitrogen atom in the sulfonamide function is sp^3 hybridized, resulting in a conformationally distinct and more flexible linker that enables lipophilic interactions of the thiophene residue with Ser97, Gly24, Val69, and the CH_2 of Asp96, while the large biphenyl residues of **4i** point away from this shallow binding pocket. Interestingly, this orientation of **4i** is in alignment with the previously observed binding pose of **II** with crystal contacts. The superimposition of **4i** and **II** suggests that the introduction of a CH_2 linker next to the carbonyl of the amide functionality of **4i** could allow a certain flexibility of the aromatic substituent and perhaps restore the lipophilic interactions in **4i** as observed for dimethylthiophene **II**.

In summary, β-fucosyl biphenylamide **4i** aligns optimally with the surface of LecB because its amide nitrogen atom is ideally positioned to form a hydrogen bond with Ser22 or an ion−dipole interaction with Asp96, its amide carbonyl oxygen interacts with Ser23, and its proximal phenyl ring forms hydrophobic interactions in the adjacent pocket with Gly24 and Val69.

Metabolic Stability, Plasma Protein Binding, and Cytotoxicity. Next, we evaluated the compounds' metabolic stability using murine/human plasma and liver microsomes and analyzed their plasma protein binding (PPB) capacity (Tables 3 and 4). In mouse plasma, 7 out of 15 compounds showed good stability with half-lives between 85 and 145 min, and 6 further compounds were fully stable: α-fucosyl benzamide (**6**), simple and aromatic β-fucosyl amides (**4b**, **4d**, **4k**, **4g**), and sulfonamide **8a**. Only compound **4f** with a *para*-chlorophenyl was less stable ($t_{1/2}$ = 23 min). In human plasma, all compounds were very stable except fucosyl sulfonamide **8b** that degraded slowly ($t_{1/2}$ = 112 min). Exceptions were acetamide **4j** and 2-furanoyl amide **4n**, which were not detectable in human and murine plasma, suggesting fast degradation.

For mouse plasma, the lowest protein binding of 21% was obtained for isopropyl amide **4k**. Bulky β-fucosyl amide **4i** as well as 2-thiophenyl sulfonamide **8b** showed 100% binding, while other fucosyl amides showed PPB between 24 and 76%. *meta*-Methylbenzamide **4h** showed only 45% binding in mouse plasma compared to its *para*-isomer **4c** with nondetectable binding due to degradation processes in mouse plasma. In

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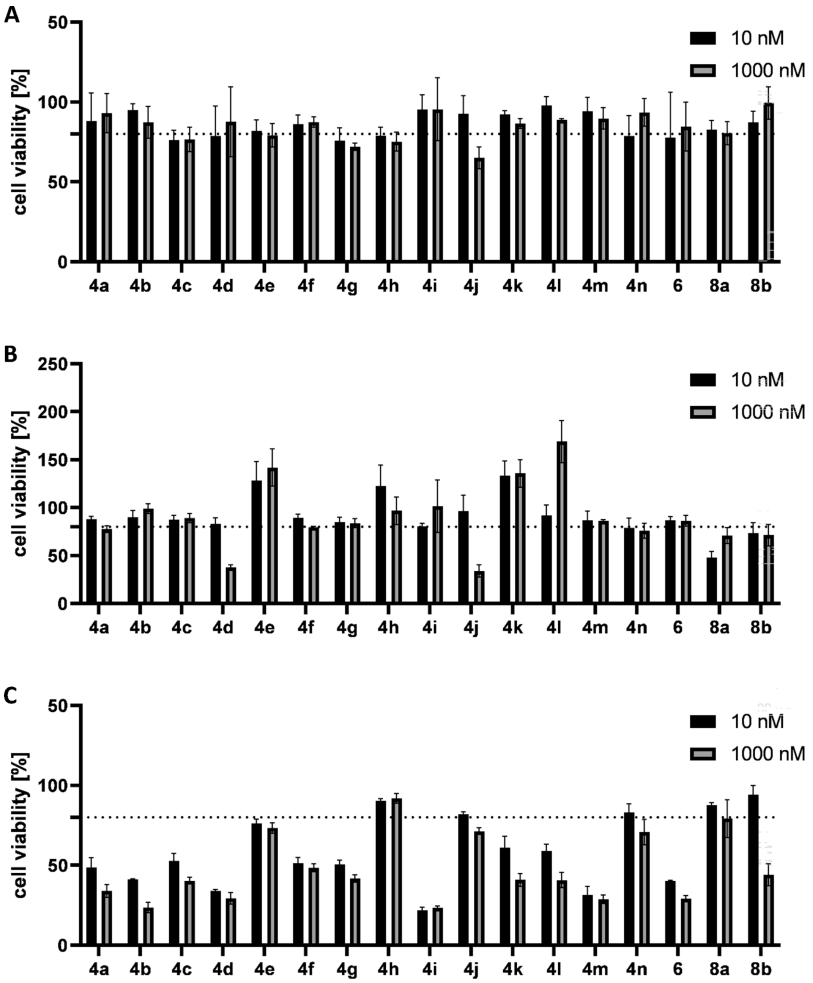

Figure 4. Cytotoxicity of α-/β-fucosyl amides and sulfonamides against the (A) lung epithelial cells A549, (B) Chinese hamster ovary cells (CHO), and (C) liver epithelial cells (HepG2) at 10 and 1000 nM concentration of LecB ligands. Cells treated with vehicle only (DMSO diluted in PBS, final DMSO concentration in the cell assay: 0.1%) served as a negative control indicated as the dashed line. The pure medium (DMEM +10% FCS) and completely damaged cells served as positive controls. The error bars show the standard deviation of minimum three independent experiments.

human plasma, most of the compounds showed a similar binding profile as in mouse plasma. However, especially **8b** exhibited differences: whereas **8b** showed much lower binding in human plasma, full binding in mouse plasma was observed. For compound **8b**, these differences might be an artifact as **8b** was less stable in plasma *per se*, with better signals in human plasma. Biphenyl amide **4i** exhibited the highest plasma protein binding in both species compared to all other tested fucosyl amides. Since the PPB directly influences the concentration of the unbound fraction of the molecules in plasma and only those are available for their therapeutic targets, the data obtained are promising for most fucosyl amides due to their moderate PPB (24−76%) and their additional metabolic stability.[56]

Then, the compounds were tested for their stability in the presence of mouse or human liver microsomes (Table 4). Most compounds exhibited a rather low clearance in the presence of both mouse microsomes (11/17 compounds, CL_{int} (m) < 23 μL min^{-1} mg^{-1} [protein]) and human liver microsomes (10/17 compounds, CL_{int} (h) < 23 μL min^{-1} mg^{-1} [protein]). *meta*-Methylbenzamide **4h** gave a moderate clearance with mouse microsomes (CL_{int} (m, **4h**) = 30 μL min^{-1} mg^{-1} [protein]) and low clearance with human liver microsomes (CL_{int} (h, **4h**) = 10 μL min^{-1} mg^{-1} [protein]). In contrast, its *para*-methyl isomer **4c** showed high clearance with microsomes of both species (CL_{int} (m, **4c**) = 61 μL min^{-1} mg^{-1} [protein], CL_{int} (h, **4c**) = 120 μL min^{-1} mg^{-1} [protein]). Interestingly, the two most potent inhibitors, **4a** and **4i**, behaved completely differently. While benzamide **4a** resulted in the highest intrinsic clearance with mouse and the second highest clearance with human liver microsomes (CL_{int} (mouse, **4a**) = 179 μL min^{-1} mg^{-1} [protein], CL_{int} (human, **4a**) = 58.4 μL min^{-1} mg^{-1} [protein]), biphenyl **4i** showed low clearance (CL_{int} (**4i**) < 23 μL min^{-1} mg^{-1} [protein]) in the presence of microsomes from both species. In general, several tested compounds had a good metabolic stability in the presence of mouse or human microsomes *in vitro* with $t_{1/2}$ > 60 min, except for the *para*/*meta*-methyl benzamides **4c** and **4 h**, unsubstituted benzamide **4a**, and naphthyl **4 m**. Nitrophenyl **4e**, thiophenyl **4b**, and furanyl **4n** were moderately stable with half-lives between $t_{1/2}$ = 47 and 56 min in mouse and $t_{1/2}$ = 25 and 60 min in human liver microsomes.

Furthermore, the cytotoxicity of α-/β-fucosyl amides and sulfonamides was assessed *in vitro* using three different cell lines, i.e., epithelial lung cell line (A549), Chinese hamster ovary cells (CHO), and epithelial liver cell line (HepG2)

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J. Med. Chem. 2022, 65, 14180−14200

(Figure 4). All tested LecB ligands displayed no toxicity against A549 cells at 10 and 1000 nM. One exception was obtained for the amide derivative **4j** with slightly reduced viability at 1 μM. Testing against CHO also revealed no toxicity for most of the tested compounds. However, a noticeable dose-dependent toxicity was observed in the case of the two amide derivatives: **4d** and **4j**. Finally, the testing against HepG2 liver epithelial cells resulted in heterogeneous cytotoxicity across our compounds. Eleven out of 15 fucosyl amides showed detectable cytotoxicity with in part strong reduction of cellular viability. Four exceptions devoid of detectable cytotoxicity against HepG2 cells were para-nitrophenyl amide **4e**, meta-methylphenyl amide **4h**, 2-furanoyl amide **4n**, as well as acetamide **4j**, a compound that was however somewhat toxic against the other two cell lines, A549 and CHO cells. No cytotoxicity against HepG2 cells was detected for sulfonamide **8a**, while sulfonamide **8b** displayed significant cytotoxicity at 1000 nM.

In summary, all fucosyl amides were sufficiently stable in human plasma, and most withstood degradation by human liver microsomes. Their intrinsic clearance was generally good except for benzamide **4a**, *para*-methylbenzamide **4c**, and 2-naphthyl **4m**. With regard to cytotoxicity, a large variation was observed between the 3 tested cell lines and the 15 tested amides. Numerous compounds showed significant cytotoxicity especially against HepG2 cells; however, some compounds did not show acute cytotoxicity in all three cell lines. This toxicity against HepG2 cells of some fucosylamides is a drawback that will require future attention, and a more detailed SAR analysis will be needed to guide the optimization.

CONCLUSIONS

In our search for novel LecB inhibitors, we have designed and synthesized fucosylmethyl thioureas as well as shortened molecules, fucosyl amides, and sulfonamides, lacking the methylene group to analyze the altered linker position on LecB binding. Surprisingly, the fucosylmethyl thioureas only showed moderate binding in the micromolar range that was also observed for the *N*-linked fucosyl sulfonamides. On the other hand, our β-fucosyl amides devoid of the methylene bridge constitute the first monovalent two-digit nanomolar LecB inhibitors with IC_{50}s of 88 nM for benzamide **4a** and 85 nM for biphenyl derivative **4i**. Noteworthily, α-fucosyl amides had been studied before with LecB, and these molecules showed moderate micromolar affinities,[53] which were confirmed by our control molecule α-benzamide **6**.

In the co-crystal structure of β-linked **4i** in complex with LecB, we demonstrated that the amide function is crucial for binding by forming a hydrogen bond with Ser22 that is located between the carbohydrate binding site and the additional subpocket, which is occupied by the proximal aromatic ring that hydrophobically interacts with Gly24 and Val69. These interactions are absent in the crystal structure of the α-anomer **6** in complex with LecB. Both crystal structures provide a basis for the interpretation of the microcalorimetric titration data for LecB with **4a** or **6**. Compounds have also been analyzed for *in vitro* early ADMET. In general, satisfying properties could be identified in all assays, except for significant cytotoxicity against one out of the three tested cell lines. Despite the fact that numerous compounds proved toxic, the presence of several derivatives without detectable cytotoxicity clearly underlines their potential for future optimization. This process should be guided by functional *in vitro* assays such as cell adhesion and biofilm formation and ultimately an infection model *in vivo*.

During the writing of this manuscript, a set of β-fucosyl amides has been reported as weak inhibitors of the *N*-terminal domain of BC2L-C, a lectin from *Burkholderia cenocepacia*,[47,48] that also binds fucose but displays a different sequence, structure, and binding site architecture compared to LecB. Despite this low affinity, further optimization could open a possibility for a molecule that potently inhibits both lectins, which could be of interest since *P. aeruginosa* and *B. cenocepacia* often co-infect cystic fibrosis patients. Thus, these β-fucosyl amides constitute a promising new class of LecB inhibitors for future use as pathoblockers against infections with *P. aeruginosa* and beyond.

EXPERIMENTAL SECTION

Chemical Synthesis. Commercial chemicals and solvents were bought at Sigma Aldrich, CarlRoth, or Carbosynth or from comparable suppliers and used without further purification. Deuterated solvents were purchased from Eurisotop (Saarbrücken, Germany). Thin layer chromatography (TLC) was performed on Silica Gel 60 coated aluminum sheets containing a fluorescence indicator (Merck KGaA, Darmstadt, Germany) and developed under UV light (254 nm) and using a molybdate solution (0.02 M solution of $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ in aqueous 10% H_2SO_4) or a potassium permanganate solution (3 g of $KMnO_4$, 20 g of K_2CO_3 in 5 mL of 5% NaOH and 300 mL of H_2O) followed by heating. For preparative medium-pressure liquid chromatography (MPLC), a Teledyne Isco Combiflash Rf200 system was used with self-packed silica gel columns (60 Å, 400 mesh particle size, Fluka, for normal-phase liquid chromatography) or prepacked Chromabond Flash RS15 C18 ec columns (60 Å, 15−40 μm particle size, Macherey-Nagel, Germany) for reversed-phase liquid chromatography. Preparative high-pressure liquid chromatography (HPLC) was performed on a Waters 2545 Binary Gradient Module equipped with a Waters 2489 UV/vis detector using a C18 column (EC HPLC column, 250/21 Nucleodur C18 Gravity SB, 5 μm particle size, Macherey-Nagel, Germany). The purity of the final test compounds was further analyzed by HPLC-UV, and all UV active compounds had a purity of at least 95%, with the exception of **8a** (purity of 87%, see Figure S2). A Thermo Dionex Ultimate 3000 HPLC (Thermo Scientific, Germany) coupled to a Bruker amaZon SL mass spectrometer equipped with a UV detector (254 nm) was used for analytical HPLC−MS. HPLC was operated with a C18 column (EC HPLC column, 100/2 Nucleoshell RP18plus, 2.7 μm particle size, Macherey-Nagel, Germany) as a stationary phase. LCMS-grade distilled CH_3CN and double distilled H_2O were used as mobile phases containing formic acid (0.1%, v/v). NMR spectroscopy was performed on a Bruker Avance III 500 UltraShield spectrometer at 500 MHz (^{1}H) or 126 MHz (^{13}C). Chemical shifts (δ) are given in parts per million (ppm), and the recorded spectra were referenced to the respective solvent peak as internal standard:[57] chloroform-d_1 (^{1}H NMR δ = 7.26 ppm, ^{13}C NMR δ = 77.0 ppm), methanol-d_4 (^{1}H NMR δ = 3.31 ppm, ^{13}C NMR δ = 49.0 ppm), and DMSO-d_6 (^{1}H NMR δ = 2.50 ppm, ^{13}C NMR δ = 39.51 ppm). Multiplicities were specified as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad signal). The signals were assigned with the help of ^{1}H,^{1}H COSY and ^{1}H,^{13}C HSQC experiments. Assignment numbering of the fucoside atoms and groups corresponds to the numbering in fucose. High-resolution mass spectra (HRMS) were recorded using an Ultimate 3000 UPLC system coupled to a Q Exactive Focus Orbitrap spectrometer with a HESI source (Thermo Fisher, Dreieich, Germany). The UPLC was operated with a C18 column (EC 150/2 Nucleodur C18 Pyramid, 3 μm particle size, Macherey-Nagel, Germany).

General Procedure (i) for Staudinger Ligation. To a solution of glycosyl azide (1.0 equiv) in dry CH_2Cl_2 (c = 0.08 M), triphenylphosphine (1.2 equiv) was added at once. The resulting

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J. Med. Chem. 2022, 65, 14180−14200

mixture was stirred at r.t. for 30 min. Then, the solution was cooled down to 0 °C, and triethylamine (1.2−2.0 equiv) was added followed by acyl chloride (1.2−2.0 equiv). The reaction mixture was slowly allowed to warm up and stirred at r.t. for 24 h−5 days until full conversion (monitored by TLC or HPLC−MS). After evaporation of the solvent, the crude material was dissolved in a minimal amount of CH_3CN, treated with H_2O (∼ 5.0 mL/1 mmol of the product), and stirred at r.t. until completion of the intermediate hydrolysis. Then, the mixture was poured into CH_2Cl_2 and washed with ice-cold satd. aq. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The obtained crude product was prepurified by flash chromatography on SiO_2 (petroleum ether/EtOAc). Further column chromatography on a C18 column (H_2O/CH_3CN) afforded the β-anomer of the protected glycosyl amide as a white solid.

General Procedure (ii) for Staudinger Ligation. To a solution of glycosyl azide (1.0 equiv) in dry CH_3CN (c = 0.08 M), triphenylphosphine (1.2 equiv) was added at once. The resulting mixture was stirred at r.t. for 30 min. Then, the solution was cooled down to 0 °C, and triethylamine (1.2−2.0 equiv) was added followed by acyl chloride (1.2−2.0 equiv). The reaction mixture was slowly allowed to warm up and stirred at r.t. for 14−48 h until full conversion (monitored by TLC or HPLC−MS). To the resulting mixture, H_2O (∼5.0 mL/1 mmol of the product) was added, and the solution was stirred at r.t. until completion of the intermediate hydrolysis. Then, the mixture was poured into CH_2Cl_2 and washed with ice-cold satd. aq. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The obtained crude product was prepurified by flash chromatography on SiO_2 (petroleum ether/EtOAc). Further column chromatography on a C18 column (H_2O/CH_3CN) afforded the β-anomer of the protected glycosyl amide as a white solid.

General Procedure (iii) for the Deacetylation of Fucopyranosyl Amides. A solution of the protected glycosyl amide (1.0 equiv) in dry CH_3OH (c = 0.05 M) was cooled down to −15 to −10 °C. Then, 0.06 M solution of $NaOCH_3$ in CH_3OH (0.1 equiv) was added dropwise, and the reaction was stirred at the same temperature for 1−2 h until no starting material was left (monitored by TLC). After completion, the pH was adjusted to pH = 7 by the addition of Amberlite IR-120 (H^+) that was removed afterward by filtration. The solvent was evaporated, and the obtained crude product was purified by flash chromatography on a C18 column (H_2O/CH_3CN), yielding the glycosyl amide as a white solid.

General Procedure (iv) for *N*-Glycosylation. To a solution of glycosyl donor (1 equiv) in dry CH_3CN (c = 0.25 M), sulfonamide acceptor (2 equiv) was added followed by dropwise addition of $BF_3{\cdot}Et_2O$ (2 equiv). The reaction mixture was stirred at r.t. for 24 h until full conversion (monitored by TLC or HPLC−MS). After completion, the solution was poured into CH_2Cl_2 and washed with ice-cold satd. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, and the solvent was evaporated. The obtained residue was purified by flash chromatography on a C18 column (4:1 → 1:1 H_2O/CH_3CN), yielding the α/β mixture of the protected glycosyl sulfonamide as a white solid. The β-anomer was isolated by crystallization from a mixture of petroleum ether/EtOAc (2:1, v/v).

General Procedure (v) for the Deacetylation of Fucopyranosyl Sulfonamides. A solution of the protected glycosyl sulfonamide (1.0 equiv) in dry CH_3OH (c = 0.03 M) was cooled down to 0 °C or −25 to −15 °C. Then, a solution of $NaOCH_3$ in CH_3OH (0.1−1.0 equiv) was added dropwise, and the reaction was stirred at the same temperature for 1.5 h or overnight until no starting material was left (monitored by TLC). After completion, the pH was adjusted to pH = 7 by addition of Amberlite IR-120 (H^+), which was removed afterward by filtration. The solvent was evaporated, and the obtained crude product was purified by flash chromatography on a C18 column (H_2O/CH_3CN) or on silica (CH_2Cl_2/MeOH) followed by HPLC (H_2O/CH_3CN), yielding the glycosyl sulfonamide as a white solid.

General Procedure (vi) for the Synthesis of Fucopyranosylmethyl Thioureas. A solution of isothiocyanate (1.0−1.1 equiv) in dry CH_3OH (c = 0.20 M) was cooled down to 0 °C. Then, a solution of β-L-fucopyranosyl methylamine (1.0 equiv) in dry CH_3OH (c = 0.20 M) was added dropwise, and the reaction mixture was stirred at 0 °C for 30 min. Subsequently, the resulting solution was slowly allowed to warm up and stirred at r.t. overnight. After completion, the solvent was evaporated, and the obtained crude product was purified by flash chromatography on SiO_2 (CH_2Cl_2/CH_3OH), yielding the fucopyranosyl thiourea as a white/pale-yellow solid.

*1,2,3,4-Tetra-O-acetyl-α/β-L-fucopyranose (**1**).* Ac_2O (8.07 mL, 85.4 mmol) was added to a solution of L-fucose (2.02 g, 12.3 mmol) in dry pyridine (20.0 mL) at 0 °C. To the resulting mixture, 4-(dimethylamino)pyridine (0.15 g, 1.23 mmol) was added. The reaction was slowly allowed to warm up and stirred at r.t. for 2.5 h. After completion, the reaction mixture was diluted with EtOAc (200 mL) and washed with 1 M aq. HCl (3 × 200 mL), satd. aq. $NaHCO_3$ (2 × 200 mL), and brine (200 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness to give a mixture of anomers (α/β = 95:5) of the desired compound **1** (4.07 g, 12.3 mmol, 99%) as a yellow syrup. The NMR data of compound **1** were consistent with the previously reported spectra.[58] The compound was prepared following the slightly modified experimental procedure from the literature.[58]

*2,3,4-Tri-O-acetyl-β-L-fucopyranosyl Azide (**2**).* Trimethylsilyl azide (0.91 mL, 6.84 mmol) was added to a solution of **1** (2.01 g, 6.05 mmol) in dry CH_2Cl_2 (13.8 mL) at 0 °C. To the resulting mixture, $SnCl_4$ (0.12 mL, 1.03 mmol) was added dropwise. The reaction was slowly allowed to warm up and stirred at r.t. for 2.5 h. After completion, the reaction mixture was diluted with CH_2Cl_2 (150 mL) and washed with satd. aq. $NaHCO_3$ (2 × 100 mL) and brine (100 mL). The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The obtained crude product was purified by flash chromatography on SiO_2 (19:1 → 13:7 petroleum ether/EtOAc), yielding the β-anomer **2** (1.74 g, 5.51 mmol, 91%) as a white solid. The NMR data of compound **2** were consistent with the previously reported spectra.[59] The compound was prepared following the experimental procedure from the literature.[59]

*S-(Pyridin-2-yl) Benzothioate (**S1**).* Benzoic acid (214 mg, 1.75 mmol) was dissolved in dry CH_3CN (5.8 mL). To the obtained solution, triphenylphosphine (689 mg, 2.63 mmol) was added followed by 2,2′-dithiodipyridine (425 mg, 1.93 mmol). The reaction mixture was stirred at 82 °C for 3 h. After completion, the solvent was evaporated, and the crude product was purified by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc), yielding compound **S1** (335 mg, 1.56 mmol, 89%) as a yellow solid. The NMR data of compound **S1** were consistent with the previously reported spectra.[60] The compound was prepared following the slightly modified experimental procedure from the literature.[60]

*Benzenesulfonamide (**S2**).* To a 25% aq. solution of NH_4OH (12.8 mL, 80.3 mmol) and CH_3OH (5.0 mL), phenylsulfonyl chloride (0.6 mL, 4.53 mmol) was added dropwise. The reaction mixture was stirred at 50 °C for 2 h. After completion, the solution was cooled down to r.t. and poured into EtOAc (300 mL). The organic layer was washed with H_2O (200 mL) and brine (200 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford compound **S2** (701 mg, 4.47 mmol, 99%) as a white solid. The obtained compound was used in the next step without further purification. The NMR data of compound **S2** were consistent with the previously reported spectra.[61]

*Thiophene-2-sulfonamide (**S3**).* To a 25% aq. solution of NH_4OH (7.8 mL, 49.0 mmol), thiophene-2-sulfonyl chloride (780 mg, 4.27 mmol) was added dropwise. The reaction mixture was stirred at 50 °C for 15 min. After completion, the solution was cooled down to r.t. and poured into EtOAc (300 mL). The organic layer was washed with H_2O (200 mL) and brine (200 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford compound **S3** (660 mg, 4.01 mmol, 94%) as a pale-yellow solid. The obtained compound was used in the next step without further purification. The NMR data of compound **S3** were consistent with the previously reported spectra.[62] The compound was prepared following the slightly modified experimental procedure from the literature.[62]

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J. Med. Chem. 2022, 65, 14180−14200

*β-L-Fucopyranosyl Nitromethane (**9**).* To a stirred solution of L-fucose (5.0 g, 30.5 mmol) and CH_3NO_2 (20.0 mL, 366 mmol) in dry DMSO (30.0 mL), 1 M solution of $NaOCH_3$ in CH_3OH (0.3 mL, 6.09 mmol) was added dropwise. The resulting solution was stirred at r.t. for 6 h. After this time, the reaction mixture was poured onto ice-cold 10 mM aq. HCl (600 mL), and the pH was adjusted to pH = 4 by addition of 1 M aq. HCl. The obtained solution was heated under reflux for 10 h. Then, the mixture was cooled down to r.t. and treated with 1 M aq. NaOH to pH = 6. After lyophilization, the residue was purified by flash chromatography on SiO_2 (99:1 → 17:3 CH_2Cl_2/CH_3OH), yielding compound **9** (2.9 g, 14.0 mmol, 46%) as a pale-yellow solid. The NMR data of compound **9** were consistent with the previously reported spectra.[63] The compound was prepared following the slightly modified experimental procedure from the literature.[64]

*β-L-Fucopyranosyl Methylamine (**10**).* A suspension of **9** (1.0 g, 4.83 mmol) and 5% Pt-C (111 mg, 0.03 mmol) in CH_3OH (43.0 mL) was stirred under a H_2 atmosphere at r.t. for 48 h. The resulting mixture was filtered over Celite, and the solvent was removed under reduced pressure to afford compound **10** (0.9 g, 4.80 mmol, 99%) as a white solid. The obtained compound was used in the next step without further purification. The NMR data of compound **10** were consistent with the previously reported spectra.[36] The compound was prepared following the slightly modified experimental procedure from the literature.[36]

*N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-benzamide (**3a**).* Compound **3a** was synthesized from **2** (225 mg, 0.71 mmol) following general procedure (i) and was obtained as a white solid (131 mg, 0.33 mmol, 47%) after purification by flash chromatography on SiO_2 (19:1 → 1:1 petroleum ether/EtOAc) and chromatography on a C18 column (4:1 → 2:3 H_2O/CH_3CN). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80−7.75 (m, 2H, 2x ArH), 7.56−7.51 (m, 1H, ArH), 7.48−7.42 (m, 2H, 2x ArH), 7.06 (br d, J = 8.7 Hz, 1H, *NH*), 5.39 (br t, J = 8.7 Hz, 1H, H-1), 5.35−5.32 (m, 1H, H-4), 5.27−5.19 (m, 2H, H-2, H-3), 4.06−4.00 (m, 1H, H-5), 2.19 (s, 3H, CH_3^{Ac}), 2.04 (s, 3H, CH_3^{Ac}), 2.02 (s, 3H, CH_3^{Ac}), 1.22 (d, J = 6.2 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.15 ($C{=}O^{Ac}$), 170.60 ($C{=}O^{Ac}$), 170.02 ($C{=}O^{Ac}$), 167.25 ($C{=}O^{Amide}$), 133.06 (ArC), 132.45 (ArCH), 128.87 (2x ArCH), 127.33 (2x ArCH), 79.20 (C-1), 71.29 (C-3), 71.01 (C-5), 70.58 (C-4), 68.78 (C-2), 21.01 (CH_3^{Ac}), 20.80 (CH_3^{Ac}), 20.77 (CH_3^{Ac}), 16.26 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3a** $[C_{19}H_{23}NO_8 + H]^+$ 394.1496; found 394.1493.

*N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-thiophene-2-carboxamide (**3b**).* Compound **3b** was synthesized from **2** (224 mg, 0.71 mmol) following general procedure (i) and was obtained as a white solid (167 mg, 0.38 mmol, 53%) after purification by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc) and chromatography on a C18 column (4:1 → 2:3 H_2O/CH_3CN). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, J = 4.9 Hz, 1H, ArH), 7.49 (d, J = 3.8 Hz, 1H, ArH), 7.08 (dd, J = 4.9, 3.8 Hz, 1H, ArH), 6.94 (d, J = 8.9 Hz, 1H, *NH*), 5.36−5.29 (m, 2H, H-1, H-4), 5.24−5.16 (m, 2H, H-2, H-3), 4.01 (br q, J = 6.4 Hz, 1H, H-5), 2.19 (s, 3H, CH_3^{Ac}), 2.04 (s, 3H, CH_3^{Ac}), 2.02 (s, 3H, CH_3^{Ac}), 1.22 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.22 ($C{=}O^{Ac}$), 170.59 ($C{=}O^{Ac}$), 169.99 ($C{=}O^{Ac}$), 161.77 ($C{=}O^{Amide}$), 137.86 (ArC), 131.63 (ArCH), 129.15 (ArCH), 127.98 (ArCH), 79.20 (C-1), 71.22 (C-3), 70.99 (C-5), 70.55 (C-4), 68.72 (C-2), 21.01 (CH_3^{Ac}), 20.80 (CH_3^{Ac}), 20.76 (CH_3^{Ac}), 16.24 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3b** $[C_{17}H_{21}NO_8S + H]^+$ 400.1061; found 400.1058.

*N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-4-methylbenzamide (**3c**).* Compound **3c** was synthesized from **2** (237 mg, 0.75 mmol) following general procedure (i) and was obtained as a white solid (204 mg, 0.49 mmol, 65%) after purification by flash chromatography on SiO_2 (17:3 → 11:9 petroleum ether/EtOAc) and column chromatography on a C18 column (9:1 → 3:2 H_2O/CH_3CN). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, J = 8.0 Hz, 2H, 2x ArH), 7.23 (d, J = 8.0 Hz, 2H, 2x ArH), 7.02 (d, J = 8.8 Hz, 1H, *NH*), 5.38 (t, J = 8.8 Hz, 1H, H-1), 5.32 (dd, J = 2.9, 0.9 Hz, 1H, H-4), 5.26−5.17 (m, 2H, H-2, H-3), 4.02 (qd, J = 6.3, 0.9 Hz, 1H, H-5), 2.39 (s, 3H, CH_3^{Ts}), 2.18 (s, 3H, CH_3^{Ac}), 2.03 (s, 3H, CH_3^{Ac}), 2.01 (s, 3H, CH_3^{Ac}), 1.21 (d, J = 6.3 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.09 ($C{=}O^{Ac}$), 170.59 ($C{=}O^{Ac}$), 170.00 ($C{=}O^{Ac}$), 167.20 ($C{=}O^{Amide}$), 143.00 (ArC), 130.21 (ArC), 129.50 (2x ArCH), 127.35 (2x ArCH), 79.17 (C-1), 71.31 (C-3), 70.96 (C-5), 70.59 (C-4), 68.75 (C-2), 21.64 (CH_3^{Ts}), 20.98 (CH_3^{Ac}), 20.78 (CH_3^{Ac}), 20.76 (CH_3^{Ac}), 16.25 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3c** $[C_{20}H_{25}NO_8 + H]^+$ 408.1653; found 408.1650.

*N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-4-methoxybenzamide (**3d**).* Compound **3d** was synthesized from **2** (224 mg, 0.71 mmol) following general procedure (i) and was obtained as a white solid (115 mg, 0.27 mmol, 38%) after purification by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc) and chromatography on a C18 column (9:1 → 3:2 H_2O/CH_3CN). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, J = 8.7 Hz, 2H, 2x ArH), 6.97 (d, J = 8.8 Hz, 1H, *NH*), 6.93 (d, J = 8.7 Hz, 2H, 2x ArH), 5.37 (t, J = 8.8 Hz, 1H, H-1), 5.33 (br d, J = 2.7 Hz, 1H, H-4), 5.25−5.18 (m, 2H, H-2, H-3), 4.02 (br q, J = 6.4 Hz, 1H, H-5), 3.85 (s, 3H, OCH_3), 2.19 (s, 3H, CH_3^{Ac}), 2.03 (s, 3H, CH_3^{Ac}), 2.02 (s, 3H, CH_3^{Ac}), 1.22 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.20 ($C{=}O^{Ac}$), 170.61 ($C{=}O^{Ac}$), 170.02 ($C{=}O^{Ac}$), 166.73 ($C{=}O^{Amide}$), 162.94 (ArC), 129.30 (2x ArCH), 125.33 (ArC), 114.05 (2x ArCH), 79.24 (C-1), 71.32 (C-3), 70.93 (C-5), 70.62 (C-4), 68.80 (C-2), 55.57 (OCH_3), 21.02 (CH_3^{Ac}), 20.81 (CH_3^{Ac}), 20.78 (CH_3^{Ac}), 16.27 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3d** $[C_{20}H_{25}NO_9 + H]^+$ 424.1602; found 424.1597.

*N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-4-nitrobenzamide (**3e**).* Compound **3e** was synthesized from **2** (228 mg, 0.72 mmol) following general procedure (i) and was obtained as a white solid (167 mg, 0.38 mmol, 53%) after purification by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc) and column chromatography on a C18 column (9:1 → 3:2 H_2O/CH_3CN). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (d, J = 8.7 Hz, 2H, 2x ArH), 7.94 (d, J = 8.7 Hz, 2H, 2x ArH), 7.22 (d, J = 8.6 Hz, 1H, *NH*), 5.41−5.29 (m, 2H, H-1, H-4), 5.26−5.16 (m, 2H, H-2, H-3), 4.03 (br q, J = 6.4 Hz, 1H, H-5), 2.18 (s, 3H, CH_3^{Ac}), 2.06 (s, 3H, CH_3^{Ac}), 2.03 (s, 3H, CH_3^{Ac}), 1.22 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.46 ($C{=}O^{Ac}$), 170.49 ($C{=}O^{Ac}$), 169.94 ($C{=}O^{Ac}$), 165.19 ($C{=}O^{Amide}$), 150.19 (ArC), 138.52 (ArC), 128.57 (2x ArCH), 124.10 (2x ArCH), 79.31 (C-1), 71.17 (C-5), 71.05 (C-3), 70.41 (C-4), 68.94 (C-2), 21.03 (CH_3^{Ac}), 20.76 (CH_3^{Ac}), 20.74 (CH_3^{Ac}), 16.22 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3e** $[C_{19}H_{22}N_2O_{10}$ -H$]^-$ 437.1202; found 437.1203.

*N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-4-chlorobenzamide (**3f**).* Compound **3f** was synthesized from **2** (236 mg, 0.75 mmol) following general procedure (ii) and was obtained as a white solid (153 mg, 0.36 mmol, 48%) after purification by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc) and column chromatography on a C18 column (4:1 → 2:3 H_2O/CH_3CN). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, J = 8.6 Hz, 2H, 2x ArH), 7.42 (d, J = 8.6 Hz, 2H, 2x ArH), 7.05 (d, J = 8.8 Hz, 1H, *NH*), 5.40−5.31 (m, 2H, H-1, H-4), 5.24−5.17 (m, 2H, H-2, H-3), 4.02 (br q, J = 6.4 Hz, 1H, H-5), 2.19 (s, 3H, CH_3^{Ac}), 2.05 (s, 3H, CH_3^{Ac}), 2.02 (s, 3H, CH_3^{Ac}), 1.22 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.31 ($C{=}O^{Ac}$), 170.56 ($C{=}O^{Ac}$), 169.99 ($C{=}O^{Ac}$), 166.16 ($C{=}O^{Amide}$), 138.83 (ArC), 131.44 (ArC), 129.18 (2x ArCH), 128.78 (2x ArCH), 79.26 (C-1), 71.19 (C-3), 71.05 (C-5), 70.52 (C-4), 68.85 (C-2), 21.02 (CH_3^{Ac}), 20.79 (CH_3^{Ac}), 20.77 (CH_3^{Ac}), 16.25 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3f** $[C_{19}H_{22}ClNO_8 + H]^+$ 428.1107; found 428.1105.

*N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-4-trifluoromethylbenzamide (**3g**).* Compound **3g** was synthesized from **2** (227 mg, 0.72 mmol) following general procedure (ii) and was obtained as a white solid (122 mg, 0.27 mmol, 37%) after purification by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc) and column chromatography on a C18 column (4:1 → 2:3 H_2O/CH_3CN). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, J = 8.2 Hz, 2H, 2x ArH), 7.72 (d, J = 8.2 Hz, 2H, 2x ArH), 7.15 (d, J = 8.8 Hz, 1H, *NH*), 5.41−5.2 (m, 2H, H-1, H-4), 5.25−5.18 (m, 2H, H-2, H-3), 4.03 (br q, J = 6.3 Hz, 1H, H-5), 2.19 (s, 3H, CH_3^{Ac}), 2.05 (s, 3H, CH_3^{Ac}), 2.03 (s, 3H, CH_3^{Ac}), 1.23 (d, J = 6.3 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.36 ($C{=}O^{Ac}$), 170.54 ($C{=}O^{Ac}$), 169.98

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J. Med. Chem. 2022, 65, 14180−14200

($C=O^{Ac}$), 165.91 ($C=O^{Amide}$), 136.31 (ArC), 134.09 (q, J = 32.9 Hz, C-CF_3), 127.83 (2x ArCH), 125.96 (q, J = 3.9 Hz, 2x ArCH), 123.69 (q, J = 272.9 Hz, CF_3), 79.28 (C-1), 71.13 (C-3, C-5), 70.48 (C-4), 68.89 (C-2), 21.01 (CH_3^{Ac}), 20.79 (CH_3^{Ac}), 20.76 (CH_3^{Ac}), 16.25 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3g** $[C_{20}H_{22}F_3NO_8 + H]^+$ 462.1370; found 462.1368.

N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-3-methylbenzamide (3h). Compound **3h** was synthesized from **2** (225 mg, 0.71 mmol) following general procedure (i) and was obtained as a white solid (132 mg, 0.33 mmol, 46%) after purification by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc) and column chromatography on a C18 column (4:1 → 2:3 H_2O/CH_3CN). ^1H NMR (500 MHz, $CDCl_3$) δ 7.61 (s, 1H, ArH), 7.52 (d, J = 6.8 Hz, 1H, ArH), 7.36−7.29 (m, 2H, 2x ArH), 7.00 (d, J = 8.9 Hz, 1H, *NH*), 5.39 (t, J = 8.9 Hz, 1H, H-1), 5.34−5.31 (m, 1H, H-4), 5.27−5.16 (m, 2H, H-2, H-3), 4.02 (br q, J = 6.4 Hz, 1H, H-5), 2.40 (s, 3H, CH_3^{Ar}), 2.19 (s, 3H, CH_3^{Ac}), 2.04 (s, 3H, CH_3^{Ac}), 2.02 (s, 3H, CH_3^{Ac}), 1.22 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.02 ($C=O^{Ac}$), 170.60 ($C=O^{Ac}$), 170.02 ($C=O^{Ac}$), 167.45 ($C=O^{Amide}$), 138.74 (ArC), 133.19 (ArCH), 133.07 (ArC), 128.71 (ArCH), 128.18 (ArCH), 124.17 (ArCH), 79.14 (C-1), 71.34 (C-3), 71.01 (C-5), 70.60 (C-4), 68.75 (C-2), 21.51 (CH_3^{Ar}), 20.99 (CH_3^{Ac}), 20.80 (CH_3^{Ac}), 20.78 (CH_3^{Ac}), 16.26 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3h** $[C_{20}H_{25}NO_8 + H]^+$ 408.1653; found 408.1650.

N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-[1,1′-biphenyl]-3-carboxamide (3i). Compound **3i** was synthesized from **2** (236 mg, 0.75 mmol) following general procedure (i) and was obtained as a white solid (94.8 mg, 0.20 mmol, 27%) after purification by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc) and column chromatography on a C18 column (4:1 → 2:3 H_2O/CH_3CN). ^1H NMR (500 MHz, $CDCl_3$) δ 8.05 (s, 1H, ArH), 7.76 (d, J = 7.8 Hz, 1H, ArH), 7.70 (d, J = 7.8 Hz, 1H, ArH), 7.65−7.60 (m, 2H, 2x ArH), 7.52 (t, J = 7.8 Hz, 1H, ArH), 7.50−7.44 (m, 2H, 2x ArH), 7.39 (t, J = 7.5 Hz, 1H, ArH), 7.12 (d, J = 8.9 Hz, 1H, *NH*), 5.42 (br t, J = 8.9 Hz, 1H, H-1), 5.36−5.33 (m, 1H, H-4), 5.28−5.20 (m, 2H, H-2, H-3), 4.04 (br q, J = 6.4 Hz, 1H, H-5), 2.19 (s, 3H, CH_3^{Ac}), 2.05 (s, 3H, CH_3^{Ac}), 2.03 (s, 3H, CH_3^{Ac}), 1.23 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.12 ($C=O^{Ac}$), 170.60 ($C=O^{Ac}$), 170.02 ($C=O^{Ac}$), 167.22 ($C=O^{Amide}$), 141.94 (ArC), 140.09 (ArC), 133.69 (ArC), 131.03 (ArCH), 129.31 (ArCH), 129.08 (2x ArCH), 127.99 (ArCH), 127.28 (2x ArCH), 126.31 (ArCH), 125.80 (ArCH), 79.23 (C-1), 71.29 (C-3), 71.04 (C-5), 70.58 (C-4), 68.84 (C-2), 21.03 (CH_3^{Ac}), 20.80 (CH_3^{Ac}), 20.77 (CH_3^{Ac}), 16.27 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3i** $[C_{25}H_{27}NO_8 + H]^+$ 470.1809; found 470.1806.

*The isolated compound contained 6% of the unknown impurity.

N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-acetamide (3j). Compound **3j** was synthesized from **2** (229 mg, 0.73 mmol) following general procedure (i) and was obtained as a white solid (104 mg, 0.31 mmol, 43%) after purification by flash chromatography on SiO_2 (19:1 → 1:4 petroleum ether/EtOAc) and column chromatography on a C18 column (9:1 → 7:3 H_2O/CH_3CN). ^1H NMR (500 MHz, $CDCl_3$) δ 6.28 (d, J = 9.2 Hz, 1H, *NH*), 5.28 (dd, J = 3.2, 1.1 Hz, 1H, H-4), 5.18 (dd, J = 9.2, 8.8 Hz, 1H, H-1), 5.12 (dd, J = 10.1, 3.2 Hz, 1H, H-3), 5.07 (dd, J = 10.1, 8.8 Hz, 1H, H-2), 3.93 (qd, J = 6.4, 1.1 Hz, 1H, H-5), 2.16 (s, 3H, CH_3^{Ac}), 2.06 (s, 3H, CH_3^{Ac}), 1.99 (s, 3H, CH_3^{Ac}), 1.98 (s, 3H, CH_3^{Amide}), 1.19 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.66 ($C=O^{Ac}$), 170.54 ($C=O^{Ac}$), 170.47 ($C=O^{Ac}$), 169.97 ($C=O^{Amide}$), 78.51 (C-1), 71.35 (C-3), 70.90 (C-5), 70.46 (C-4), 68.57 (C-2), 23.54 (CH_3^{Amide}), 20.94 (CH_3^{Ac}), 20.76 (CH_3^{Ac}), 20.73 (CH_3^{Ac}), 16.22 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3j** $[C_{14}H_{21}NO_8 + H]^+$ 332.1340; found 332.1336.

N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-isobutyramide (3k). Compound **3k** was synthesized from **2** (230 mg, 0.73 mmol) following general procedure (i) and was obtained as a white solid (32.2 mg, 0.09 mmol, 12%) after purification by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc) and column chromatography on a C18 column (9:1 → 7:3 H_2O/CH_3CN). ^1H NMR (500 MHz, $CDCl_3$) δ 6.26 (d, J = 9.0 Hz, 1H, *NH*), 5.28 (dd, J = 3.0, 1.1 Hz, 1H, H-4), 5.19 (t, J = 9.0 Hz, 1H, H-1), 5.14−5.06 (m, 2H, H-2, H-3), 3.93 (qd, J = 6.4, 1.1 Hz, 1H, H-5), 2.38−2.28 (m, 1H, CH^{iPr}), 2.16 (s, 3H, CH_3^{Ac}), 2.03 (s, 3H, CH_3^{Ac}), 1.98 (s, 3H, CH_3^{Ac}), 1.18 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}), 1.12 (d, J = 7.1 Hz, 3H, CH_3^{iPr}), 1.10 (d, J = 7.5 Hz, 3H, CH_3^{iPr}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.20 ($C=O^{Ac}$), 171.60 ($C=O^{Ac}$), 170.56 ($C=O^{Ac}$), 169.97 ($C=O^{Amide}$), 78.46 (C-1), 71.31 (C-3), 70.98 (C-5), 70.52 (C-4), 68.64 (C-2), 35.69 (CH^{iPr}), 20.88 (CH_3^{Ac}), 20.76 (CH_3^{Ac}), 20.72 (CH_3^{Ac}), 19.58 (CH_3^{iPr}), 18.86 (CH_3^{iPr}), 16.21 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3k** $[C_{16}H_{25}NO_8 + H]^+$ 360.1653; found 360.1648.

N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-cinnamamide (3l). Compound **3l** was synthesized from **2** (226 mg, 0.72 mmol) following general procedure (i) and was obtained as a white solid (199 mg, 0.48 mmol, 66%) after purification by flash chromatography on SiO_2 (19:1 → 13:11 petroleum ether/EtOAc) and column chromatography on a C18 column (4:1 → 2:3 H_2O/CH_3CN). ^1H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, J = 15.6 Hz, 1H, =CH), 7.53−7.47 (m, 2H, 2x ArH), 7.41−7.35 (m, 3H, 3x ArH), 6.45 (d, J = 9.0 Hz, 1H, *NH*), 6.33 (d, J = 15.6 Hz, 1H, =CH), 5.38−5.29 (m, 2H, H-1, H-4), 5.20−5.14 (m, 2H, H-2, H-3), 3.99 (br q, J = 6.4 Hz, 1H, H-5), 2.18 (s, 3H, CH_3^{Ac}), 2.05 (s, 3H, CH_3^{Ac}), 2.01 (s, 3H, CH_3^{Ac}), 1.21 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.85 ($C=O^{Ac}$), 170.58 ($C=O^{Ac}$), 169.99 ($C=O^{Ac}$), 166.00 ($C=O^{Amide}$), 143.16 (=CH), 134.44 (ArC), 130.33 (ArCH), 129.03 (2x ArCH), 128.17 (2x ArCH), 119.67 (=CH), 78.79 (C-1), 71.37 (C-3), 70.96 (C-5), 70.54 (C-4), 68.65 (C-2), 20.98 (CH_3^{Ac}), 20.78 (CH_3^{Ac}), 20.74 (CH_3^{Ac}), 16.26 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3l** $[C_{21}H_{25}NO_8 + H]^+$ 420.1653; found 420.1648.

N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-2-naphthamide (3m). Compound **3m** was synthesized from **2** (213 mg, 0.67 mmol) following general procedure (ii) and was obtained as a white solid (98.4 mg, 0.22 mmol, 33%) after purification by flash chromatography on SiO_2 (9:1 → 3:2 petroleum ether/EtOAc) and column chromatography on a C18 column (4:1 → 2:3 H_2O/CH_3CN). ^1H NMR (500 MHz, $CDCl_3$) δ 8.31 (s, 1H, ArH), 7.96 (d, J = 8.0 Hz, 1H, ArH), 7.91 (d, J = 8.8 Hz, 1H, ArH), 7.88 (d, J = 8.0 Hz, 1H, ArH), 7.82 (d, J = 8.8 Hz, 1H, ArH), 7.62−7.53 (m, 2H, 2x ArH), 7.21 (d, J = 9.1 Hz, 1H, *NH*), 5.46 (br t, J = 9.1 Hz, 1H, H-1), 5.38−5.34 (m, 1H, H-4), 5.32−5.26 (m, 1H, H-2), 5.26−5.21 (m, 1H, H-3), 4.06 (qd, J = 6.5, 1.8 Hz, 1H, H-5), 2.20 (s, 3H, CH_3^{Ac}), 2.05 (s, 3H, CH_3^{Ac}), 2.03 (s, 3H, CH_3^{Ac}), 1.24 (d, J = 6.5 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.16 ($C=O^{Ac}$), 170.61 ($C=O^{Ac}$), 170.04 ($C=O^{Ac}$), 167.31 ($C=O^{Amide}$), 135.24 (ArC), 132.67 (ArC), 130.27 (ArC), 129.37 (ArCH), 128.81 (ArCH), 128.32 (ArCH), 128.23 (ArCH), 127.90 (ArCH), 127.01 (ArCH), 123.52 (ArCH), 79.29 (C-1), 71.33 (C-3), 71.06 (C-5), 70.61 (C-4), 68.83 (C-2), 21.02 (CH_3^{Ac}), 20.81 (CH_3^{Ac}), 20.79 (CH_3^{Ac}), 16.29 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3m** $[C_{23}H_{25}NO_8 + H]^+$ 444.1653; found 444.1649.

N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-furan-2-carboxamide (3n). Compound **3n** was synthesized from **2** (227 mg, 0.72 mmol) following general procedure (i) and was obtained as a white solid (157 mg, 0.41 mmol, 57%) after purification by flash chromatography on SiO_2 (19:1 → 3:2 petroleum ether/EtOAc) and column chromatography on a C18 column (4:1 → 2:3 H_2O/CH_3CN). ^1H NMR (500 MHz, $CDCl_3$) δ 7.48 (d, J = 1.4 Hz, 1H, ArH), 7.16 (d, J = 3.3 Hz, 1H, ArH), 7.13 (d, J = 9.1 Hz, 1H, *NH*), 6.50 (dd, J = 3.3, 1.4 Hz, 1H, ArH), 5.34 (t, J = 9.1 Hz, 1H, H-1), 5.31 (br d, J = 3.3 Hz, 1H, H-4), 5.23 (dd, J = 10.2, 9.1 Hz, 1H, H-2), 5.17 (dd, J = 10.2, 3.3 Hz, 1H, H-3), 3.99 (br q, J = 6.3 Hz, 1H, H-5), 2.18 (s, 3H, CH_3^{Ac}), 2.02 (s, 3H, CH_3^{Ac}), 2.01 (s, 3H, CH_3^{Ac}), 1.20 (d, J = 6.3 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.51 ($C=O^{Ac}$), 170.61 ($C=O^{Ac}$), 170.03 ($C=O^{Ac}$), 158.35 ($C=O^{Amide}$), 147.02 (ArC), 145.03 (ArCH), 115.91 (ArCH), 112.42 (ArCH), 78.31 (C-1), 71.43 (C-3), 71.01 (C-5), 70.53 (C-4), 68.42 (C-2), 20.92 (CH_3^{Ac}), 20.79 (CH_3^{Ac}), 20.76 (CH_3^{Ac}), 16.25 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **3n** $[C_{17}H_{21}NO_9 + H]^+$ 384.1289; found 384.1287.

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*N-(2,3,4-Tri-O-acetyl-α-L-fucopyranosyl)-benzamide (**5**).* To a solution of **2** (153 mg, 0.49 mmol) and 4 Å molecular sieves in dry CH_3NO_2 (5.5 mL), triphenylphosphine (140 mg, 0.53 mmol) was added at once. The resulting mixture was stirred at 100 °C for 24 h. Then, the solution was cooled down to r.t., and **S1** (136 mg, 0.63 mmol) was added followed by $CuCl_2$ (84.9 mg, 0.63 mmol). The reaction mixture was heated up and stirred at 40 °C for 24 h. After this time, the reaction mixture was filtered through Celite. The obtained filtrate was diluted with CH_2Cl_2 (100 mL) and washed with H_2O (50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The obtained crude product was prepurified by flash chromatography on SiO_2 (9:1 → 3:2 petroleum ether/EtOAc). Further column chromatography on a C18 column (4:1 → 3:2 H_2O/CH_3CN) afforded the compound **5** (51.7 mg, 0.13 mmol, 27%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, J = 8.0 Hz, 2H, 2x ArH), 7.56 (br t, J = 7.5 Hz, 1H, ArH), 7.48 (dd, J = 8.0, 7.5 Hz, 2H, 2x ArH), 6.85 (br d, J = 7.5 Hz, 1H, *NH*), 6.07 (dd, J = 7.5, 5.6 Hz, 1H, H-1), 5.49 (dd, J = 10.3, 5.6 Hz, 1H, H-2), 5.35−5.24 (m, 2H, H-3, H-4), 4.11 (br q, J = 6.4 Hz, 1H, H-5), 2.20 (s, 3H, CH_3^{Ac}), 2.03 (s, 3H, CH_3^{Ac}), 2.00 (s, 3H, CH_3^{Ac}), 1.19 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.83 ($C{=}O^{Ac}$), 170.65 ($C{=}O^{Ac}$), 169.48 ($C{=}O^{Ac}$), 168.12 ($C{=}O^{Amide}$), 133.73 (ArC), 132.48 (ArCH), 128.96 (2x ArCH), 127.39 (2x ArCH), 75.33 (C-1), 70.62 (C-4), 68.34 (C-3), 66.23 (C-2), 65.83 (C-5), 20.82 (2x CH_3^{Ac}), 20.80 (CH_3^{Ac}), 16.23 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **5** $[C_{19}H_{23}NO_8 + H]^+$ 394.1496; found 394.1494.

*N-(β-L-Fucopyranosyl)-benzamide (**4a**).* Compound **4a** was synthesized from **3a** (42.9 mg, 0.11 mmol) following general procedure (iii) and was obtained as a white solid (26.7 mg, 0.10 mmol, 94%) after purification by flash chromatography on a C18 column (19:1 H_2O/CH_3CN), yielding the compound **4a** (26.7 mg, 0.10 mmol, 94%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.92−7.88 (m, 2H, 2x ArH), 7.56 (t, J = 7.4 Hz, 1H, ArH), 7.47 (br t, J = 7.4 Hz, 2H, 2x ArH), 5.07 (d, J = 9.0 Hz, 1H, H-1), 3.78 (qd, J = 6.5, 1.0 Hz, 1H, H-5), 3.72−3.66 (m, 2H, H-2, H-4), 3.58 (dd, J = 9.5, 3.3 Hz, 1H, H-3), 1.27 (d, J = 6.5 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 170.95 (C=O), 135.43 (ArC), 133.01 (ArCH), 129.51 (2x ArCH), 128.69 (2x ArCH), 82.03 (C-1), 76.00 (C-3), 73.81 (C-5), 73.26 (C-4), 70.95 (C-2), 16.89 (CH_3) ppm. HR-MS (ESI): calcd for **4a** $[C_{13}H_{17}NO_5 + H]^+$ 268.1179; found 268.1176.

*N-(β-L-Fucopyranosyl)-thiophene-2-carboxamide (**4b**).* Compound **4b** was synthesized from **3b** (41.9 mg, 0.10 mmol) following general procedure (iii) and was obtained as a white solid (25.3 mg, 0.09 mmol, 88%) after purification by flash chromatography on a C18 column (199:1 → 19:5 H_2O/CH_3CN). ^{1}H NMR (500 MHz, CD_3OD) δ 7.82 (dd, J = 3.8, 1.1 Hz, 1H, ArH), 7.69 (dd, J = 5.0, 1.1 Hz, 1H, ArH), 7.14 (dd, J = 5.0, 3.8 Hz, 1H, ArH), 5.02 (d, J = 9.0 Hz, 1H, H-1), 3.76 (qd, J = 6.4, 1.0 Hz, 1H, H-5), 3.72−3.65 (m, 2H, H-2, H-4), 3.57 (dd, J = 9.5, 3.3 Hz, 1H, H-3), 1.26 (d, J = 6.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 164.98 (C=O), 139.96 (ArC), 132.46 (ArCH), 130.43 (ArCH), 128.82 (ArCH), 81.96 (C-1), 75.99 (C-3), 73.78 (C-5), 73.23 (C-4), 70.86 (C-2), 16.88 (CH_3) ppm. HR-MS (ESI): calcd for **4b** $[C_{11}H_{15}NO_5S + Na]^+$ 296.0563; found 296.0560.

*N-(β-L-Fucopyranosyl)-4-methylbenzamide (**4c**).* Compound **4c** was synthesized from **3c** (73.1 mg, 0.18 mmol) following general procedure (iii) and was obtained as a white solid (49.3 mg, 0.18 mmol, 98%) after purification by flash chromatography on a C18 column (199:1 → 19:1 H_2O/CH_3CN). ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (d, J = 8.1 Hz, 2H, 2x ArH), 7.29 (d, J = 8.1 Hz, 2H, 2x ArH), 5.06 (d, J = 9.0 Hz, 1H, H-1), 3.78 (br q, J = 6.5, 1H, H-5), 3.71−3.65 (m, 2H, H-2, H-4), 3.57 (dd, J = 9.5, 3.4 Hz, 1H, H-3), 2.40 (s, 3H, CH_3^{Ar}), 1.26 (d, J = 6.5 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 170.89 (C=O), 143.78 (ArC), 132.54 (ArC), 130.11 (2x ArCH), 128.75 (2x ArCH), 82.01 (C-1), 76.01 (C-3), 73.78 (C-5), 73.27 (C-4), 70.96 (C-2), 21.46 (CH_3^{Ar}), 16.89 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **4c** $[C_{14}H_{19}NO_5 + H]^+$ 282.1336; found 282.1332.

*N-(β-L-Fucopyranosyl)-4-methoxybenzamide (**4d**).* Compound **4d** was synthesized from **3d** (55.9 mg, 0.13 mmol) following general procedure (iii) and was obtained as a white solid (35.0 mg, 0.12 mmol, 89%) after purification by flash chromatography on a C18 column (199:1 → 19:1 H_2O/CH_3CN). ^{1}H NMR (500 MHz, CD_3OD) δ 7.88 (d, J = 8.9 Hz, 2H, 2x ArH), 6.99 (d, J = 8.9 Hz, 2H, 2x ArH), 5.05 (d, J = 9.0 Hz, 1H, H-1), 3.85 (s, 3H, OCH_3), 3.77 (qd, J = 6.5, 1.1 Hz, 1H, H-5), 3.71−3.65 (m, 2H, H-2, H-4), 3.58 (dd, J = 9.6, 3.3 Hz, 1H, H-3), 1.26 (d, J = 6.5 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 170.45 (C=O), 164.25 (ArC), 130.65 (2x ArCH), 127.37 (ArC), 114.68 (2x ArCH), 82.04 (C-1), 76.01 (C-3), 73.74 (C-5), 73.28 (C-4), 70.97 (C-2), 55.94 (OCH_3), 16.89 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **4d** $[C_{14}H_{19}NO_6 + H]^+$ 298.1285; found 298.1282.

*N-(β-L-Fucopyranosyl)-4-nitrobenzamide (**4e**).* Compound **4e** was synthesized from **3e** (55.9 mg, 0.13 mmol) following general procedure (iii) and was obtained as a white solid (49.9 mg, 0.16 mmol, 99%) after purification by flash chromatography on a C18 column (199:1 H_2O/CH_3CN). ^{1}H NMR (500 MHz, CD_3OD) δ 8.33 (d, J = 8.8 Hz, 2H, 2x ArH), 8.10 (d, J = 8.8 Hz, 2H, 2x ArH), 5.08 (d, J = 9.0 Hz, 1H, H-1), 3.79 (qd, J = 6.5, 1.1 Hz, 1H, H-5), 3.73−3.67 (m, 2H, H-2, H-4), 3.59 (dd, J = 9.5, 3.3 Hz, 1H, H-3), 1.27 (d, J = 6.5 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 168.89 (C=O), 151.23 (ArC), 141.22 (ArC), 130.09 (2x ArCH), 124.56 (2x ArCH), 82.05 (C-1), 75.95 (C-3), 73.94 (C-5), 73.20 (C-4), 70.94 (C-2), 16.89 (CH_3) ppm. HR-MS (ESI): calcd for **4e** $[C_{13}H_{16}N_2O_7 - H]^-$ 311.0885; found 311.0887.

*N-(β-L-Fucopyranosyl)-4-chlorobenzamide (**4f**).* Compound **4f** was synthesized from **3f** (42.6 mg, 0.10 mmol) following general procedure (iii) and was obtained as a white solid (21.6 mg, 0.07 mmol, 72%) after purification by flash chromatography on a C18 column (19:1 → 9:1 H_2O/CH_3CN). ^{1}H NMR (500 MHz, CD_3OD) δ 7.89 (d, J = 8.6 Hz, 2H, 2x ArH), 7.49 (d, J = 8.6 Hz, 2H, 2x ArH), 5.05 (d, J = 9.2 Hz, 1H, H-1), 3.78 (br q, J = 6.5 Hz, 1H, H-5), 3.72−3.62 (m, 2H, H-2, H-4), 3.57 (dd, J = 9.4, 3.4 Hz, 1H, H-3), 1.26 (d, J = 6.5 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 169.73 (C=O), 139.09 (ArC), 134.09 (ArC), 130.43 (2x ArCH), 129.70 (2x ArCH), 82.04 (C-1), 75.98 (C-3), 73.85 (C-5), 73.23 (C-4), 70.94 (C-2), 16.89 (CH_3) ppm. HR-MS (ESI): calcd for **4f** $[C_{13}H_{16}ClNO_5 - H]^-$ 300.0644; found 300.0645.

*N-(β-L-Fucopyranosyl)-4-trifluoromethylbenzamide (**4g**).* Compound **4g** was synthesized from **3g** (40.6 mg, 0.09 mmol) following general procedure (iii) and was obtained as a white solid (27.1 mg, 0.09 mmol, 99%) after purification by flash chromatography on a C18 column (19:1 → 9:1 H_2O/CH_3CN). ^{1}H NMR (500 MHz, CD_3OD) δ 8.07 (d, J = 8.3 Hz, 2H, 2x ArH), 7.79 (d, J = 8.3 Hz, 2H, 2x ArH), 5.08 (d, J = 9.1 Hz, 1H, H-1), 3.78 (qd, J = 6.5, 1.1 Hz, 1H, H-5), 3.73−3.68 (m, 2H, H-2, H-4), 3.59 (dd, J = 9.5, 3.4 Hz, 1H, H-3), 1.27 (d, J = 6.5 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 169.51 (C=O), 139.17 (ArC), 134.30 (q, J = 32.6 Hz, $\underline{C}$-CF_3), 129.48 (2x ArCH), 126.46 (q, J = 3.7 Hz, 2x ArCH), 125.29 (q, J = 271.1 Hz, CF_3), 82.03 (C-1), 75.95 (C-3), 73.89 (C-5), 73.22 (C-4), 70.93 (C-2), 16.89 (CH_3) ppm. HR-MS (ESI): calcd for **4 g** $[C_{14}H_{16}F_3NO_5 - H]^-$ 334.0908; found 334.0909.

*N-(β-L-Fucopyranosyl)-3-methylbenzamide (**4h**).* Compound **4h** was synthesized from **3h** (40.6 mg, 0.10 mmol) following general procedure (iii) and was obtained as a white solid (20.4 mg, 0.07 mmol, 73%) after purification by flash chromatography C18 silica gel on (95:5 H_2O/CH_3CN). ^{1}H NMR (500 MHz, CD_3OD) δ 7.73 (br s, 1H, ArH), 7.68 (br d, J = 7.4 Hz, 1H, ArH), 7.40−7.32 (m, 2H, 2x ArH), 5.06 (d, J = 9.1 Hz, 1H, H-1), 3.78 (qd, J = 6.5, 1.0 Hz, 1H, H-5), 3.71−3.65 (m, 2H, H-2, H-4), 3.58 (dd, J = 9.5, 3.4 Hz, 1H, H-3), 2.41 (s, 3H, CH_3^{Ar}), 1.26 (d, J = 6.5 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 171.15 (C=O), 139.51 (ArC), 135.40 (ArC), 133.66 (ArCH), 129.44 (ArCH), 129.21 (ArCH), 125.84 (ArCH), 82.03 (C-1), 76.00 (C-3), 73.80 (C-5), 73.28 (C-4), 70.96 (C-2), 21.36 (CH_3^{Ar}), 16.89 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **4h** $[C_{14}H_{19}NO_5 + H]^+$ 282.1336; found 282.1332.

*N-(β-L-Fucopyranosyl)-[1,1′-biphenyl]-3-carboxamide (**4i**).* Compound **4i** was synthesized from **3i** (38.9 mg, 0.08 mmol) following

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J. Med. Chem. 2022, 65, 14180−14200

general procedure (iii) and was obtained as a white solid (25.4 mg, 0.07 mmol, 89%) after purification by flash chromatography on a C18 column (19:1−7:3 H_2O/CH_3CN). ^1H NMR (500 MHz, CD_3OD) δ 8.17 (t, J = 1.8 Hz, 1H, ArH), 7.88 (dt, J = 7.8, 1.4 Hz, 1H, ArH), 7.82 (dt, J = 7.8, 1.5 Hz, 1H, ArH), 7.72−7.66 (m, 2H, 2x ArH), 7.56 (t, J = 7.7 Hz, 1H, ArH), 7.50−7.44 (m, 2H, 2x ArH), 7.37 (dt, J = 7.4, 1.3 Hz, 1H, ArH), 5.10 (d, J = 9.0 Hz, 1H, H-1), 3.80 (qd, J = 6.5, 1.1 Hz, 1H, H-5), 3.75−3.67 (m, 2H, H-2, H-4), 3.59 (dd, J = 9.5, 3.4 Hz, 1H, H-3), 1.28 (d, J = 6.5 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 170.96 (C=O), 142.85 (ArC), 141.55 (ArC), 136.06 (ArC), 131.46 (ArCH), 130.10 (ArCH), 129.99 (2x ArCH), 128.83 (ArCH), 128.14 (2x ArCH), 127.58 (ArCH), 127.30 (ArCH), 82.09 (C-1), 76.00 (C-3), 73.84 (C-5), 73.28 (C-4), 70.96 (C-2), 16.91 (CH_3) ppm. HR-MS (ESI): calcd for **4i** $[C_{19}H_{21}NO_5 + H]^+$ 344.1492; found 344.1487.

*N-(β-L-Fucopyranosyl)-acetamide (**4j**).* Compound **4j** was synthesized from **3j** (45.1 mg, 0.14 mmol) following general procedure (iii) and was obtained as a white solid (26.6 mg, 0.12 mmol, 84%) after purification by flash chromatography on a C18 column (99.9:0.1 H_2O/CH_3CN). ^1H NMR (500 MHz, CD_3OD) δ 4.81 (d, J = 8.9 Hz, 1H, H-1), 3.69 (qd, J = 6.4, 1.1 Hz, 1H, H-5), 3.63 (dd, J = 2.7, 1.1 Hz, 1H, H-4), 3.53−3.46 (m, 2H, H-2, H-3), 2.00 (s, 3H, CH_3^{Ac}), 1.23 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 174.23 (C=O), 81.31 (C-1), 75.95 (C-3), 73.61 (C-5), 73.17 (C-4), 71.05 (C-2), 22.86 (CH_3^{Ac}), 16.85 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **4j** $[C_8H_{15}NO_5 + Na]^+$ 228.0842; found 228.0839.

*N-(β-L-Fucopyranosyl)-isobutyramide (**4k**).* Compound **4k** was synthesized from **3k** (32.0 mg, 0.09 mmol) following general procedure (iii) and was obtained as a white solid (17.6 mg, 0.08 mmol, 85%) after purification by flash chromatography on a C18 column (999:1 H_2O/CH_3CN). ^1H NMR (500 MHz, CD_3OD) δ 4.82 (d, J = 8.9 Hz, 1H, H-1), 3.70 (qd, J = 6.5, 1.1 Hz, 1H, H-5), 3.64 (dd, J = 2.7, 1.1 Hz, 1H, H-4), 3.54−3.48 (m, 2H, H-2, H-3), 2.53−2.44 (m, J = 6.9 Hz, 1H, CH^{iPr}), 1.23 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}), 1.14 (d, J = 6.9 Hz, 3H, CH_3^{iPr}), 1.13 (d, J = 6.9 Hz, 3H, CH_3^{iPr}) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 180.96 (C=O), 81.29 (C-1), 76.01 (C-3), 73.58 (C-5), 73.22 (C-4), 71.04 (C-2), 36.31 (CH^{iPr}), 19.69 (2x CH_3^{iPr}), 16.88 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **4k** $[C_{10}H_{19}NO_5 + Na]^+$ 256.1155; found 256.1152.

*N-(β-L-Fucopyranosyl)-cinnamamide (**4l**).* Compound **4l** was synthesized from **3l** (76.4 mg, 0.18 mmol) following general procedure (iii) and was obtained as a white solid (53.2 mg, 0.18 mmol, 99%) after purification by flash chromatography on a C18 column (19:1 → 9:1 H_2O/CH_3CN). ^1H NMR (500 MHz, CD_3OD) δ 7.62 (d, J = 15.8 Hz, 1H, =CH), 7.59−7.55 (m, 2H, 2x ArH), 7.43−7.35 (m, 3H, 3x ArH), 6.67 (d, J = 15.8 Hz, 1H, =CH), 4.97 (d, J = 8.4 Hz, 1H, H-1), 3.76 (qd, J = 6.5, 1.0 Hz, 1H, H-5), 3.67 (dd, J = 3.1, 1.0 Hz, 1H, H-4), 3.59 (dd, J = 9.4, 8.4 Hz, 1H, H-2), 3.56 (dd, J = 9.4, 3.1 Hz, 1H, H-3), 1.25 (d, J = 6.5 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 169.18 (C=O), 142.93 (=CH), 136.16 (ArC), 131.03 (ArCH), 129.97 (2x ArCH), 128.96 (2x ArCH), 121.70 (=CH), 81.57 (C-1), 75.99 (C-3), 73.71 (C-5), 73.21 (C-4), 71.16 (C-2), 16.89 (CH_3) ppm. HR-MS (ESI): calcd for **4l** $[C_{15}H_{19}NO_5 + H]^+$ 294.1336; found 294.1331.

*N-(β-L-Fucopyranosyl)-2-naphthamide (**4m**).* Compound **4m** was synthesized from **3m** (41.6 mg, 0.09 mmol) following general procedure (iii) and was obtained as a white solid (27.7 mg, 0.09 mmol, 96%) after purification by flash chromatography on a C18 column (19:1 → 9:1 H_2O/CH_3CN). ^1H NMR (500 MHz, CD_3OD) δ 8.48 (s, 1H, ArH), 7.98 (d, J = 8.0 Hz, 1H, ArH), 7.96−7.93 (m, 2H, 2x ArH), 7.91 (d, J = 8.0 Hz, 1H, ArH), 7.61−7.53 (m, 2H, 2x ArH), 5.14 (d, J = 9.1 Hz, 1H, H-1), 3.81 (qd, J = 6.5, 1.0 Hz, 1H, H-5), 3.76 (dd, J = 9.5, 9.1 Hz, 1H, H-2), 3.70 (dd, J = 3.4, 1.0 Hz, 1H, H-4), 3.62 (dd, J = 9.5, 3.4 Hz, 1H, H-3), 1.28 (d, J = 6.5 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 170.99 (C=O), 136.44 (ArC), 133.98 (ArC), 132.61 (ArC), 130.10 (ArCH), 129.33 (ArCH), 129.27 (ArCH), 128.97 (ArCH), 128.77 (ArCH), 127.84 (ArCH), 125.15 (ArCH), 82.12 (C-1), 76.00 (C-3), 73.83 (C-5), 73.28 (C-4), 71.00 (C-2), 16.91 (CH_3) ppm. HR-MS (ESI): calcd for **4m** $[C_{17}H_{19}NO_5 + H]^+$ 318.1336; found 318.1332.

*N-(β-L-Fucopyranosyl)-furan-2-carboxamide (**4n**).* Compound **4n** was synthesized from **3n** (68.3 mg, 0.18 mmol) following general procedure (iii) and was obtained as a white solid (47.1 mg, 0.15 mmol, 85%) after purification by flash chromatography on a C18 column (199:1 → 19:1 H_2O/CH_3CN). ^1H NMR (500 MHz, CD_3OD) δ 7.70 (d, J = 1.8 Hz, 1H, ArH), 7.21 (d, J = 3.5 Hz, 1H, ArH), 6.60 (dd, J = 3.5, 1.8 Hz, 1H, ArH), 5.02 (d, J = 9.1 Hz, 1H, H-1), 3.76 (qd, J = 6.4, 1.0 Hz, 1H, H-5), 3.67 (dd, J = 3.4, 1.0 Hz, 1H, H-4), 3.67 (dd, J = 9.5, 9.1 Hz, 1H, H-2), 3.56 (dd, J = 9.5, 3.4 Hz, 1H, H-3), 1.28 (d, J = 6.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 161.20 (C=O), 148.71 (ArC), 146.71 (ArCH), 116.28 (ArCH), 113.10 (ArCH), 81.38 (C-1), 75.93 (C-3), 73.83 (C-5), 73.24 (C-4), 70.93 (C-2), 16.88 (CH_3) ppm. HR-MS (ESI): calcd for **4n** $[C_{11}H_{15}NO_6 + Na]^+$ 280.0792; found 280.0788.

*N-(α-L-Fucopyranosyl)-benzamide (**6**).* Compound **6** was synthesized from **5** (19.9 mg, 0.05 mmol) following general procedure (iii) and was obtained as a white solid (11.6 mg, 0.05 mmol, 98%) after purification by flash chromatography on a C18 column (199:1 → 19:5 H_2O/CH_3CN). ^1H NMR (500 MHz, CD_3OD) δ 7.86 (d, J = 8.0 Hz, 2H, 2x ArH), 7.56 (t, J = 7.5 Hz, 1H, ArH), 7.48 (dd, J = 8.0, 7.5 Hz, 2H, 2x ArH), 5.75 (d, J = 5.6 Hz, 1H, H-1), 4.05 (dd, J = 10.3, 5.6 Hz, 1H, H-2), 3.94−3.87 (m, 2H, H-3, H-5), 3.69 (dd, J = 3.5, 1.2 Hz, 1H, H-4), 1.22 (d, J = 6.5 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 171.85 (C=O), 135.74 (ArC), 132.95 (ArCH), 129.51 (2x ArCH), 128.73 (2x ArCH), 79.10 (C-1), 73.20 (C-4), 71.47 (C-3), 68.77 (C-5), 68.20 (C-2), 16.87 (CH_3) ppm. HR-MS (ESI): calcd for **6** $[C_{13}H_{17}NO_5 + H]^+$ 268.1179; found 268.1177.

*N-(2,3,4-Tri-O-acetyl-β-l-fucopyranosyl)benzenesulfonamide (**7a**).* Compound **7a** was synthesized from **1** (131 mg, 0.39 mmol) and **S2** (122 mg, 0.78 mmol) following general procedure (iv) and was obtained as a white solid (81.2 mg, 0.18 mmol, 47%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, J = 8.2 Hz, 2H, 2x ArH), 7.58 (t, J = 7.3 Hz, 1H, ArH), 7.50 (dd, J = 8.2, 7.3 Hz, 2H, 2x ArH), 5.46 (d, J = 9.9 Hz, 1H, NH), 5.21 (dd, J = 3.3, 1.1 Hz, 1H, H-4), 5.07 (dd, J = 10.3, 3.3 Hz, 1H, H-3), 5.02 (dd, J = 10.3, 9.9 Hz, 1H, H-2), 4.74 (dd, J = 10.0, 8.7 Hz, 1H, H-1), 3.77 (qd, J = 6.4, 1.1 Hz, 1H, H-5), 2.12 (s, 3H, CH_3^{Ac}), 1.97 (s, 3H, CH_3^{Ac}), 1.94 (s, 3H, CH_3^{Ac}), 1.00 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.22 (C=O), 170.54 (C=O), 170.01 (C=O), 141.39 (ArC), 132.98 (ArCH), 129.03 (2x ArCH), 127.22 (2x ArCH), 83.20 (C-1), 71.17 (C-3), 70.90 (C-5), 70.09 (C-4), 67.80 (C-2), 20.77 (CH_3^{Ac}), 20.75 (CH_3^{Ac}), 20.71 (CH_3^{Ac}), 15.91 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **7a** $[C_{18}H_{23}NO_9S\ -H]^-$ 428.1021; found 428.1024.

*N-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)thiophene-2-sulfonamide (**7b**).* Compound **7b** was synthesized from **1** (134 mg, 0.40 mmol) and **S3** (130 mg, 0.80 mmol) following general procedure (iv) and was obtained as a white solid (102 mg, 0.24 mmol, 59%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, J = 3.8 Hz, 1H, ArH), 7.60 (d, J = 4.9 Hz, 1H, ArH), 7.07 (dd, J = 4.9, 3.8 Hz, 1H, ArH), 5.58 (d, J = 9.8 Hz, 1H, NH), 5.24 (dd, J = 3.2, 1.1 Hz, 1H, H-4), 5.09 (dd, J = 10.2, 3.2 Hz, 1H, H-3), 5.04 (dd, J = 10.2, 9.8 Hz, 1H, H-2), 4.77 (dd, J = 10.0, 8.6 Hz, 1H, H-1), 3.80 (qd, J = 6.4, 1.1 Hz, 1H, H-5), 2.13 (s, 3H, CH_3^{Ac}), 2.00 (s, 3H, CH_3^{Ac}), 1.98 (s, 3H, CH_3^{Ac}), 1.06 (d, J = 6.4 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.23 (C=O), 170.54 (C=O), 170.00 (C=O), 142.24 (ArC), 132.72 (ArCH), 132.46 (ArCH), 127.34 (ArCH), 83.36 (C-1), 71.16 (C-3), 71.05 (C-5), 70.10 (C-4), 67.70 (C-2), 20.84 (CH_3^{Ac}), 20.77 (CH_3^{Ac}), 20.72 (CH_3^{Ac}), 16.01 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **7b** $[C_{16}H_{21}NO_9S_2\ -H]^-$ 434.0585; found 434.0588.

*N-(L-Fucopyranosyl)benzenesulfonamide (**8a**).* Compound **8a** (α/β 13:87) was synthesized from **7a** (38.9 mg, 0.09 mmol) following general procedure (v) (−25 to −15 °C; 0.1 equiv of $NaOCH_3$) and was obtained as a white solid (20.3 mg, 0.07 mmol, 75%) after purification by flash chromatography on a C18 column (199:1 → 9:1 H_2O/CH_3CN). Main β-anomer: ^1H NMR (500 MHz, CD_3OD) δ 7.90 (d, J = 8.0 Hz, 2H, 2x ArH), 7.57 (t, J = 7.5 Hz, 1H, ArH), 7.50 (dd, J = 8.0, 7.5 Hz, 2H, 2x ArH), 4.41 (d, J = 8.7 Hz, 1H, H-1), 3.54 (br d, J = 3.3 Hz, 1H, H-4), 3.48 (br q, J = 6.4 Hz, 1H, H-

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J. Med. Chem. 2022, 65, 14180−14200

5), 3.45 (dd, J = 9.6, 3.3 Hz, 1H, H-3), 3.40 (dd, J = 9.6, 8.7 Hz, 1H, H-2), 0.93 (d, J = 6.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 144.06 (ArC), 133.28 (ArCH), 129.57 (2x ArCH), 128.28(2x ArCH), 86.50 (C-1), 75.80 (C-3), 73.19 (C-5), 72.89 (C-4), 71.06 (C-2), 16.40 (CH_3) ppm. HR-MS (ESI): calcd for **8a** $[C_{12}H_{17}NO_6S - H]^-$ 302.0704; found 302.0704.

*N-(L-Fucopyranosyl)thiophene-2-sulfonamide (**8b**).* Compound **8b** (α/β 10:90) was synthesized from **7b** (88.8 mg, 0.21 mmol) following general procedure (v) (0 °C; 1.0 equiv of $NaOCH_3$) and was obtained as a white solid (12.5 mg, 0.04 mmol, 19%) after purification by HPLC (199:1 → 4:1 CH_2Cl_2/CH_3OH). Main β-anomer: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.66 (s, 1H, *NH*), 7.85 (dd, J = 5.0, 1.4 Hz, 1H, ArH), 7.59 (dd, J = 3.7, 1.4 Hz, 1H, ArH), 7.11 (dd, J = 5.0, 3.7 Hz, 1H, ArH), 4.81 (d, J = 4.2 Hz, 1H, *OH*), 4.74 (d, J = 4.8 Hz, 1H, *OH*), 4.45 (d, J = 4.0 Hz, 1H, *OH*), 4.30 (d, J = 8.5 Hz, 1H, H-1), 3.45 (br q, J = 6.5 Hz, 1H, H-5), 3.39 (br dd, J = 4.0, 2.4 Hz, 1H, H-4), 3.33−3.28 (m, 2H, H-2, H-3), 0.94 (d, J = 6.5 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 144.04 (ArC), 131.77 (ArCH), 131.52 (ArCH), 126.97 (ArCH), 85.27 (C-1), 74.24 (C-3), 71.37 (C-5), 70.83 (C-4), 68.91 (C-2), 16.31 (CH_3) ppm. HR-MS (ESI): calcd for **8b** $[C_{10}H_{15}NO_6S_2$ -H$]^-$ 308.0268; found 308.0268.

*1-(β-L-Fucopyranosylmethyl)-3-(4-methylphenyl)thiourea (**11c**).* Compound **11c** was synthesized from 4-methylphenyl isothiocyanate (76.4 mg, 0.51 mmol) and **10** (90.7 mg, 0.51 mmol) following general procedure (vi) and was obtained as a white solid (113 mg, 0.36 mmol, 71%) after purification by flash chromatography on SiO_2 (199:1 → 4:1 CH_2Cl_2/CH_3OH). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.63 (s, 1H, *NH*), 7.40 (br s, 1H, *NH*), 7.30 (d, J = 8.3 Hz, 2H, 2x ArH), 7.12 (d, J = 8.3 Hz, 2H, 2x ArH), 4.85 (br s, 1H, *OH*), 4.66 (d, J = 5.0 Hz, 1H, *OH*), 4.40 (d, J = 4.7 Hz, 1H, *OH*), 3.98 (br d, J = 13.4 Hz, 1H, CH_2), 3.52 (br q, J = 6.5 Hz, 1H, H-5), 3.46 (br dd, J = 4.7, 1.6 Hz, 1H, H-4), 3.42−3.35 (m, 1H, CH_2), 3.33−3.25 (m, 2H, H-2, H-3), 3.21−3.11 (m, 1H, H-1), 2.26 (s, 3H, CH_3^{Ar}), 1.13 (d, J = 6.5 Hz, 3H, CH_3^{Fuc}) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.24 (C=S), 136.59 (ArC), 133.35 (ArC), 129.10 (2x ArCH), 123.07 (2x ArCH), 77.82 (C-1), 74.49 (C-3), 73.79 (C-5), 71.50 (C-4), 68.61 (C-2), 46.13 (CH_2), 20.48 (CH_3^{Ar}), 17.07 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **11c** $[C_{15}H_{22}N_2O_4S + H]^+$ 327.1373; found 327.1369.

*1-(β-L-Fucopyranosylmethyl)-3-(4-methoxyphenyl)thiourea (**11d**).* Compound **11d** was synthesized from 4-methoxyphenyl isothiocyanate (70.0 μL, 0.52 mmol) and **10** (88.1 mg, 0.50 mmol) following general procedure (vi) and was obtained as a white solid (135 mg, 0.40 mmol, 79%) after purification by flash chromatography on SiO_2 (199:1 → 17:3 CH_2Cl_2/CH_3OH). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.53 (s, 1H, *NH*), 7.32−7.20 (m, 3H, *NH*, 2x ArH), 6.89 (d, J = 8.9 Hz, 2H, 2x ArH), 4.85 (br s, 1H, *OH*), 4.66 (d, J = 5.1 Hz, 1H, *OH*), 4.40 (d, J = 4.7 Hz, 1H, *OH*), 3.95 (br d, J = 13.5 Hz, 1H, CH_2), 3.73 (s, 3H, OCH_3), 3.51 (br q, J = 6.4 Hz, 1H, H-5), 3.46 (br dd, J = 4.7, 1.7 Hz, 1H, H-4), 3.42−3.35 (m, 1H, CH_2), 3.34−3.24 (m, 2H, H-2, H-3), 3.20−3.10 (m, 1H, H-1), 1.12 (d, J = 6.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.58 (C=S), 156.38 (ArC), 131.81 (ArC), 125.40 (2x ArCH), 113.90 (2x ArCH), 77.83 (C-1), 74.49 (C-3), 73.79 (C-5), 71.50 (C-4), 68.62 (C-2), 55.25 (OCH_3), 46.17 (CH_2), 17.06 (CH_3^{Fuc}) ppm. HR-MS (ESI): calcd for **11d** $[C_{15}H_{22}N_2O_5S + H]^+$ 343.1322; found 343.1319.

*1-(β-L-Fucopyranosylmethyl)-3-(4-nitrophenyl)thiourea (**11e**).* Compound **11e** was synthesized from 4-nitrophenyl isothiocyanate (94.1 mg, 0.52 mmol) and **10** (89.0 mg, 0.50 mmol) following general procedure (vi) and was obtained as a pale-yellow solid (125 mg, 0.36 mmol, 71%) after purification by flash chromatography on SiO_2 (199:1 → 17:3 CH_2Cl_2/CH_3OH). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.34 (s, 1H, *NH*), 8.17 (d, J = 9.2 Hz, 2H, 2x ArH), 8.09 (br s, 1H, *NH*), 7.89 (d, J = 9.2 Hz, 2H, 2x ArH), 4.89 (d, J = 3.8 Hz, 1H, *OH*), 4.69 (d, J = 5.0 Hz, 1H, *OH*), 4.43 (d, J = 4.9 Hz, 1H, *OH*), 4.04 (br d, J = 13.7 Hz, 1H, NCH_2), 3.54 (br q, J = 6.4 Hz, 1H, H-5), 3.48 (br dd, J = 4.9, 1.5 Hz, 1H, H-4), 3.43−3.35 (m, 1H, NCH_2), 3.35−3.27 (m, 2H, H-2, H-3), 3.25−3.18 (m, 1H, H-1), 1.17 (d, J = 6.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 179.84 (C=S), 146.45 (ArC), 141.71 (ArC), 124.54 (2x ArCH), 120.10 (2x ArCH), 77.55 (C-1), 74.55 (C-3), 73.83 (C-5), 71.54 (C-4), 68.55 (C-2), 46.18 (CH_2), 17.11 (CH_3) ppm. HR-MS (ESI): calcd for **11e** $[C_{14}H_{19}N_3O_6S - H]^-$ 356.0922; found 356.0922.

*1-(β-L-Fucopyranosylmethyl)-3-(4-ethylphenyl)thiourea (**11o**).* Compound **11o** was synthesized from 4-ethylphenyl isothiocyanate (80.0 μL, 0.51 mmol) and **10** (85.3 mg, 0.48 mmol) following general procedure (vi) and was obtained as a white solid (122 mg, 0.36 mmol, 74%) after purification by flash chromatography on SiO_2 (199:1 → 17:3 CH_2Cl_2/CH_3OH). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.65 (s, 1H, *NH*), 8.31 (s, $CDCl_3$), 7.40 (br s, 1H, *NH*), 7.33 (d, J = 8.4 Hz, 2H, 2x ArH), 7.15 (d, J = 8.4 Hz, 2H, 2x ArH), 4.85 (d, J = 3.5 Hz, 1H, *OH*), 4.66 (d, J = 5.0 Hz, 1H, *OH*), 4.40 (d, J = 4.7 Hz, 1H, *OH*), 3.99 (br d, J = 13.6 Hz, 1H, NCH_2), 3.52 (br q, J = 6.4 Hz, 1H, H-5), 3.46 (br dd, J = 4.7, 1.8 Hz, 1H, H-4), 3.41−3.34 (m, 1H, NCH_2), 3.32−3.27 (m, 2H, H-2, H-3), 3.20−3.13 (m, 1H, H-1), 2.57 (q, J = 7.6 Hz, 2H, CH_2^{Et}), 1.16 (t, J = 7.6 Hz, 3H, CH_3^{Et}), 1.13 (d, J = 6.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.21 (C=S), 139.74 (ArC), 136.79 (ArC), 127.91 (2x ArCH), 123.12 (2x ArCH), 79.18 ($CDCl_3$), 77.81 (C-1), 74.49 (C-3), 73.78 (C-5), 71.49 (C-4), 68.60 (C-2), 46.11 (NCH_2), 27.62 (CH_2^{Et}), 17.05 (CH_3^{Fuc}), 15.68 (CH_3^{Et}) ppm. HR-MS (ESI): calcd for **11o** $[C_{16}H_{24}N_2O_4S +$ H$]^+$ 341.1530; found 341.1525.

*1-(β-L-Fucopyranosylmethyl)-3-(4-fluorophenyl)thiourea (**11p**).* Compound **11p** was synthesized from 4-fluorophenyl isothiocyanate (75.6 mg, 0.49 mmol) and **10** (83.3 mg, 0.47 mmol) following general procedure (vi) and was obtained as a white solid (124 mg, 0.38 mmol, 80%) after purification by flash chromatography on SiO_2 (199:1 → 17:3 CH_2Cl_2/CH_3OH). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.70 (s, 1H, *NH*), 8.31 (s, $CDCl_3$), 7.50 (br s, 1H, *NH*), 7.48−7.41 (m, 2H, 2x ArH), 7.17−7.10 (m, 2H,2x ArH), 4.86 (br s, 1H, *OH*), 4.67 (d, J = 5.0 Hz, 1H, *OH*), 4.41 (d, J = 3.5 Hz, 1H, *OH*), 3.99 (br d, J = 12.8 Hz, 1H, CH_2), 3.52 (br q, J = 6.4 Hz, 1H, H-5), 3.46 (br dd, J = 4.8, 1.7 Hz, 1H, H-4), 3.42−3.34 (m, 1H, CH_2), 3.32−3.26 (m, 2H, H-2, H-3), 3.22−3.13 (m, 1H, H-1), 1.14 (d, J = 6.4 Hz, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.65 (C=S), 158.79 (d, J = 240.7 Hz, C-F), 135.70 (ArC), 125.13 (2x ArCH), 115.12 (d, J = 22.7 Hz, 2x ArCH), 79.18 ($CDCl_3$), 77.80 (C-1), 74.51 (C-3), 73.79 (C-5), 71.51 (C-4), 68.58 (C-2), 46.14 (CH_2), 17.08 (CH_3) ppm. HR-MS (ESI): calcd for **11p** $[C_{14}H_{19}FN_2O_4S - H]^-$ 329.0977; found 329.0977.

Docking. The crystal structure coordinates of LecB in complex with fucose (PDB: 1OXC) were adjusted for docking in MOE (Molecular Operating Environment, Chemical Computing Group, Canada version: 2014.0901) by removing all ligands and water molecules and keeping one monomer. The coordinates of the carbohydrate binding site were determined in AutoDockTools[65] and added into the docking file. Four amino acid—Asn21, Glu95, Asp101, and Asn103—were kept flexible during the docking run. In parallel, ligands for docking were drawn in ChemDraw, exported as SMILES code, and translated into a pdb file using the online SMILES Translator.[66] The ligand pdb files were processed in MOE, and bonds were set to the lowest energy level and exported as a mol2 file and added to the docking file. The docking was performed with Plants1.1[67] using a binding site radius of 13 Å. For validation of the protocol, docking was performed first with α-L-fucose, and the resulting pose was then superimposed with its co-crystal structure in MOE, which confirmed the same interactions. Afterward, the two compounds, **4a** and **11c**, were docked. The results were visualized and analyzed in MOE.

Recombinant Expression and Purification of LecB. LecB from *P. aeruginosa* PAO1 was expressed and purified from *Escherichia coli* BL21 (DE3) and the plasmid pET25pa2l[68] as described before.[37]

Competitive Binding Assay. The competitive binding assay based on fluorescence polarization was performed with small modifications in analogy to the one described before.[13] To a 10 μL solution of $LecB_{PAO1}$ (150 nM) and the fluorescent reporter ligand (*N*-(fluorescein-5-yl)-*N*-(α-L-fucosyl-oxy-ethylene)-thiocarbamide, 20 nM) in TBS/Ca^{2+} buffer containing 0.02% DMSO (20 mM Tris−HCl, 137 mM NaCl, 2.6 mM KCl, 1 mM $CaCl_2$, pH = 7.4) in a black 384-well plate (Greiner Bio-One, Germany, cat. no. 781900), a 10 μL

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J. Med. Chem. 2022, 65, 14180−14200

serial dilution of the inhibitor in the same buffer (10−0.005 μM, dilution factor 2) was added (technical triplicates). The plate was sealed (EASYseal, Greiner Bio-One, cat. no. 676001), centrifuged (1500 *g*, r.t., 1 min), and incubated in a dark chamber under shaking conditions for 24 h. Afterward, the seal was removed, and fluorescence was measured on a PHERAstar FS (BMG Labtech, Germany, filter ex.: 485 nm, em.: 535 nm). After blank value (TBS/Ca^{2+} buffer with LecB) subtraction from the measured fluorescence intensities, polarization was calculated, and the data were analyzed using the four-parameter variable slope model in the MARS software (BMG Labtech). Then, top and bottom plateaus were redefined using the full inhibition value in the presence of the positive control L-fucose and full binding value for LecB and reporter ligand in the absence of inhibitor as a negative control. Three independent experiments were performed, and the data were averaged and visualized using GraphPad PRISM (version 5). Fucosylmethyl thioureas were tested at a final LecB concentration of 150 nM, a dilution series 100−0.78 μM, and 0.1% DMSO in the TBS/Ca^{2+} buffer.

Isothermal Titration Calorimetry. Compounds **4a**, **6**, and $LecB_{PAO1}$ were separately dissolved in the same TBS/Ca^{2+} buffer, and the concentration for LecB was determined by UV absorbance (ε = 6990 M^{-1} cm^{-1})[69]. Experiments were performed on a MicroCal iTC_{200} (Malvern Panalytical, United Kingdom) instrument by titrating the ligand (500−1500 μM) into the LecB solution (100 μM) with stirring (700 rpm) at 25 °C. The reference power was set to 5 μcal s^{-1}, the filter period to 5 s, and 19−39 injections (0.5−2 μL per injection) with an injection duration of 1 s and a spacing of 240 s between each injection were performed per experiment. In case of titrations with lower ligand concentrations (500 μM), the syringe was refilled after the first run ended, and the experiments were continued with the same sample cell contents to reach saturation. The resulting data files were merged with the MicroCal Concat ITC software. The first injection of every titration was discarded, and the data were analyzed with the MicroCal Origin software using the one-site binding model. ITC data are depicted in Figure 2 and Table S3.

X-ray Crystallography of $LecB_{PAO1}$ in Complex with 6 or 4i. $LecB_{PAO1}$ at 10.8 mg mL^{-1} in water with 1 mM $CaCl_2$ (**4a** and **4i**) or at 9.3 mg mL^{-1} in 20 mM Hepes pH 7.5, 100 mM NaCl, and 100 μM $CaCl_2$ (**6**) was incubated in a 19:1 ratio with 2.5 mM of compound for 30 min to 1 h prior to crystallization. Stock solutions at 50 mM of the compound were made in water for **4a** and in 100% DMSO for **4i** and **6**. The hanging drop vapor diffusion method using 1 μL of the protein−ligand mixture with 1 μL of the reservoir solution at 19 °C in a 24-well plate yielded crystals after 1−3 days. The crystals for the $LecB_{PAO1}$-**4a** or $LecB_{PAO1}$-**4i** complexes were obtained with 30 and 28% PEG 8 K, 200 mM $(NH_4)_2SO_4$, and 100 mM Tris (pH 8.5), respectively, and those for $LecB_{PAO1}$−**6** were obtained with 24% Peg8K, 1 M LiCl, and 100 mM sodium acetate (pH 4.4). All solutions were cryoprotected, and the crystal was directly mounted in a cryoloop and flash-frozen in liquid nitrogen. Diffraction data were collected at 100 K at Synchrotron SOLEIL (Paris, France) on beamline Proxima 1 using an EIGER X 16M area detector for $LecB_{PAO1}$-**4a** and $LecB_{PAO1}$-**4i** or Proxima 2 using an EIGER X 9M area detector for $LecB_{PAO1}$-**6**. The data were processed using XDS and XDSme.[70,71] All further computing was performed using the CCP4 suite.[72] Five percent of the observations were set aside for cross-validation analysis, and hydrogen atoms were added in their riding positions and used for geometry and structure-factor calculations. The structure was solved by molecular replacement using PHASER.[73] For complexes with **4i** and **6**, the coordinates of the 5A3O tetramer were used as a search model to search for one tetramer in the asymmetric unit. The structures were refined with restrained maximum likelihood refinement using REFMAC 5.8[74] iterated with manual rebuilding in Coot.[75] Ligand libraries were created using JLigand. The ligands were introduced after inspection of the 2Fo−DFc weighted maps. Water molecules, introduced automatically using Coot, were inspected manually. The stereochemical quality of the models was assessed with the PDB Validation Server. The structure of LecB in complex with **4a** was solved by molecular replacement at 2.5 Å using Phaser and a search for one tetramer and two dimers from model 1GZT. The low-resolution did not give suitable electron density, and we decided not to refine it. Data quality and refinement statistics are summarized in Supporting Information, Table S4. All structural figures were created using PyMOL. Authors will release the atomic coordinates upon article publication.

Plasma Stability Assay. Each compound, dissolved in DMSO (10 mM), was added to mouse plasma or to human plasma (pH = 7.4, 37 °C) to yield a final concentration of 1 μM. In addition, procaine and procainamide (dissolved in DMSO) were added to yield a final concentration of 1 μM. Procaine served as a positive control as it is unstable in mouse plasma. Procainamide served as a negative control as it is stable in mouse plasma. The samples were incubated for 0, 15, 30, 60, 90, 120, and 240 min at 37 °C. At each time point, 10 μL of the respective sample was extracted with 90 μL of CH_3CN and 12.5 ng/mL of caffeine as an internal standard for 5 min at 2000 rpm on a MixMate vortex mixer (Eppendorf). CH_3CN and caffeine were dispensed using a Mantis Formulatrix. Then samples were centrifuged for 20 min at 2270 *g* at 4 °C, and the supernatants were transferred to 96-well Greiner V-bottom plates and afterward measured by HPLC−MS. Peak areas of each compound and of the internal standard were analyzed using the MultiQuant 3.0 software (AB Sciex). Peak areas of the respective compound were normalized to the internal standard peak area and to the respective peak areas at time point 0 min with eq 1:

$$\mathrm{Norm}_{\text{peak area}} = \frac{C \times D^{-1}}{A \times B^{-1}} \tag{1}$$

where *A*: peak area of the compound at the time point 0 min, *B*: peak area of the internal standard at time point 0 min, *C*: peak area of the compound at the respective time point, and *D*: peak area of the internal standard at the respective time point.

***In Vitro* Metabolic Stability Assay.** Liver microsomes (mouse and human, Thermo Fisher) were thawed slowly on ice. A total of 5 mg mL^{-1} of microsomes, 2 μL of a 100 μM solution of every compound, and 183 μL of 100 mM phosphate buffer were incubated 5 min at 37 °C in a water bath. Reactions were initiated using 10 μL of 20 mM NADPH (CarlRoth, Germany). Samples were incubated in three replicates at 37 °C under gentle agitation at 150 rpm. At 0, 5, 15, 30, and 60 min, reactions were terminated by the addition of 180 μL of CH_3CN. Samples were vortexed for 5 min and then centrifuged at 2270 *g* for 20 min at 4 °C. The supernatants were transferred to 96-well Greiner V-bottom plates, sealed with WebSeal nonsterile mats (Thermo Fisher), and analyzed according to the section HPLC−MS analysis. Peak areas of the respective time point of the compounds were normalized to the peak area at time point 0 min. Then half-life was calculated using linear regression. Cl_{int} [μL/min/mg protein] was calculated using the following eq 2:

$$\mathrm{CL}_{\text{int}}[\mu\mathrm{L\ min^{-1}\ mg_{(protein)}^{-1}}] = \frac{0.693}{0.005 \times t_{1/2}} \tag{2}$$

Plasma Protein Binding. Plasma protein binding was assessed using the rapid equilibrium device (RED) system from ThermoFisher. Compounds were dissolved in DMSO to a concentration of 10 mM. Naproxene served as control as it shows high plasma protein binding. Compounds were diluted in murine plasma (from CD-1 mice, pooled, Biomol GmbH) or in human plasma (human donors, both genders, pooled, antibodies-online GmbH) to a final concentration of 1 μM. Dialysis buffer and plasma samples were added to the respective chambers according the manufacturer's protocol. The RED plate was sealed with a tape and incubated at 37 °C for 2 h at 800 rpm on an Eppendorf MixMate vortex-mixer. Then samples (dialysis and plasma samples) were withdrawn from the respective chambers. To 25 μL of each dialysis sample, 25 μL of plasma and, to 25 μL of the plasma sample, 25 μL of the dialysis buffer were added. Then, 150 μL of the ice-cold extraction solvent (CH_3CN/H_2O (90:10) containing 12.5 ng mL^{-1} caffeine as internal standard) was added. Samples were incubated for 30 min on ice. Then, samples were centrifuged at 4 °C at 2270 *g* for 10 min. Supernatants were transferred to Greiner V-

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J. Med. Chem. 2022, 65, 14180−14200

bottom 96-well plates and sealed with a tape. The percentage of bound compound was calculated with the eqs 3 and 4:

$$\%_{free} = \frac{c_{buffer\ chamber}}{c_{plasma\ chamber}} \times 100 \quad (3)$$

$$\%_{bound} = 100\% - \%_{free} \quad (4)$$

HPLC−MS Analysis. Samples were analyzed using an Agilent 1290 Infinity II HPLC system coupled to an AB Sciex QTrap 6500plus mass spectrometer. LC conditions were as follows: column: Agilent Zorbax Eclipse Plus C18, 50 × 2.1 mm, 1.8 μm; temperature: 30 °C; injection volume: 5 μL per sample; flow rate: 700 μL min^{-1}. Samples were run under acidic and buffered conditions. Solvents for acidic conditions were as follows: A1: water + 0.1% formic acid; solvent B1: 95% CH_3CN/5% H_2O + 0.1% formic acid; solvents for buffered conditions were as follows: A2: 95% H_2O + 5% CH_3CN + 5 mM ammonium acetate + 40 μL L^{-1} acetic acid; B2: 95% CH_3CN + 5% H_2O + 5 mM ammonium acetate + 40 μL L^{-1} acetic acid. The same gradient was applied for acidic and buffered conditions: 99% A at 0 min, 99% A until 1 min, 99−0% A from 1.0 to 4.0 min, and 0% A until 5.0 min. Mass transitions for controls and compounds are depicted in Table S5.

Cytotoxicity. The epithelial liver cell line HepG2 (ATCC HB-8065TM) and the epithelial lung cell line A549 (ATCC CCL-185) were cultivated in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal calf serum (FCS) at 37 °C and 5% CO_2. CHO cells (ATCC CCL-61) were cultivated in Gibco Ham's F-12K medium supplemented with 10% FCS. Cells were seeded into a 96-well plate (Nunc, Roskilde, Denmark) and grown to 75% confluency. The following compounds were tested in the cell assay: **4a−4n**, **6**, **8a**, and **8b**. Every compound was dissolved in DMSO and diluted in PBS (final DMSO concentration in the cell assay: 0.1%). Cells were incubated for 24 h at 37 °C and 5% CO_2 with the respective compound at two different concentrations (10 nM and 1 μM), allowing for a rapid screen. Cells treated with vehicle only (DMSO diluted in PBS, final DMSO concentration in the cell assay: 0.1%) served as a negative control. Furthermore, the pure medium (DMEM + 10% FCS) and completely damaged cells served as positive controls. To damage cells, cells were treated with 0.5% Triton X-100 1 h prior to addition of MTT (Sigma). After 24 h, cells were washed twice with DMEM + 10% FCS (A549 and HepG2 cells) or Ham's F-12K + 10% FCS (CHO-cells). MTT diluted in PBS (stock solution 5 mg/mL) was added to the wells at a final concentration of 1 mg/mL. The cells were incubated for 3 h at 37 °C and 5% CO_2. The medium was removed, and 0.04 M HCl in 2-propanol was added. The cells were incubated at room temperature for 15 min. Then the supernatant was transferred to a 96-well plate. UV absorbance of the samples was measured at 560 and 670 nm as a reference wavelength on a Tecan Sunrise ELISA reader using the Magellan software. Data were normalized using the following formula: $(A - B)/(C - B)$, with A as the respective data point, B as the value of the Triton X-100-treated control, and C as the vehicle control. The experiment was repeated at least three times. The error bars indicate the standard deviation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.2c01373.

^1H and ^{13}C NMR spectra of new compounds, purity of key compounds by LCMS, protein−ligand interaction maps of the docked compounds, β-fucosyl benzamide **4a** and β-fucosylmethyl thiourea **11c**, ITC data of all replicates for titrations of LecB with β-/α-fucosyl benzamides **4a** and **6**, X-ray data collection and refinement statistics of LecB complexed structures and a zoom into the electron density and interactions of the ligand with each LecB protomers, and m/z search window for plasma stability and mass transitions of the tested compounds from the ADME studies (PDF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATION

CF, cystic fibrosis; CHO, Chinese hamster ovary; CL, clearance; COPD, chronic obstructive pulmonary disease; dev., deviation; DMEM, Dulbecco's modified Eagles medium; FCS, fetal calf serum; ITC, isothermal titration calorimetry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; n.d., not detected; PPB, plasma protein binding; Ref, reference; std., standard; TBS, Tris-buffered saline; temp, temperature

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