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Comparison of affinity ranking by target-directed dynamic combinatorial chemistry and surface plasmon resonance

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Dedicated to Steven Hanessian, for his friendship and support over many years

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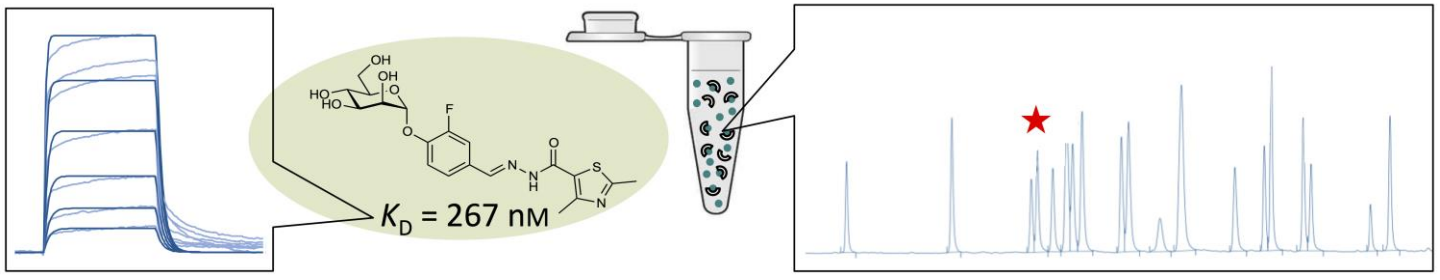
Abstract

Target-directed dynamic combinatorial chemistry (tdDCC) is a powerful method to screen ligands for pharmacologically relevant targets. Generating a dynamic library from reversibly reacting building blocks in the presence of a target protein leads to the amplification of the most potent library constituents. In previous studies on tdDCC, these compounds were identified in a qualitative “hit/no-hit”-manner. However, the precise relationship between the degree of amplification and the affinity of the library constituent has not yet been evaluated. To study the amplification–affinity relationship, we compared tdDCC experiments, employing reversible acylhydrazone formation and the bacterial adhesin FimH as a target, with affinities of the library constituents as determined by surface plasmon resonance.



Keywords: Acylhydrazone, FimH antagonists, dynamic combinatorial chemistry, supramolecular chemistry, drug discovery

Introduction

Dynamic combinatorial chemistry (DCC) describes the generation of dynamic compound libraries from reversibly reacting building blocks. These libraries, which are under thermodynamic control, remain adaptive by continuous interconversion of building blocks and products. Therefore, addition of a protein target alters their equilibrium composition by binding, thereby stabilizing, and ultimately amplifying specific library constituents. Target-directed DCC (tdDCC) exhibits a self-screening ability, leading to the amplification of those members of the library with the highest affinity for the protein target, as depicted for an acylhydrazone library in Figure 1. This makes tdDCC a valuable tool for drug discovery.[1-6]

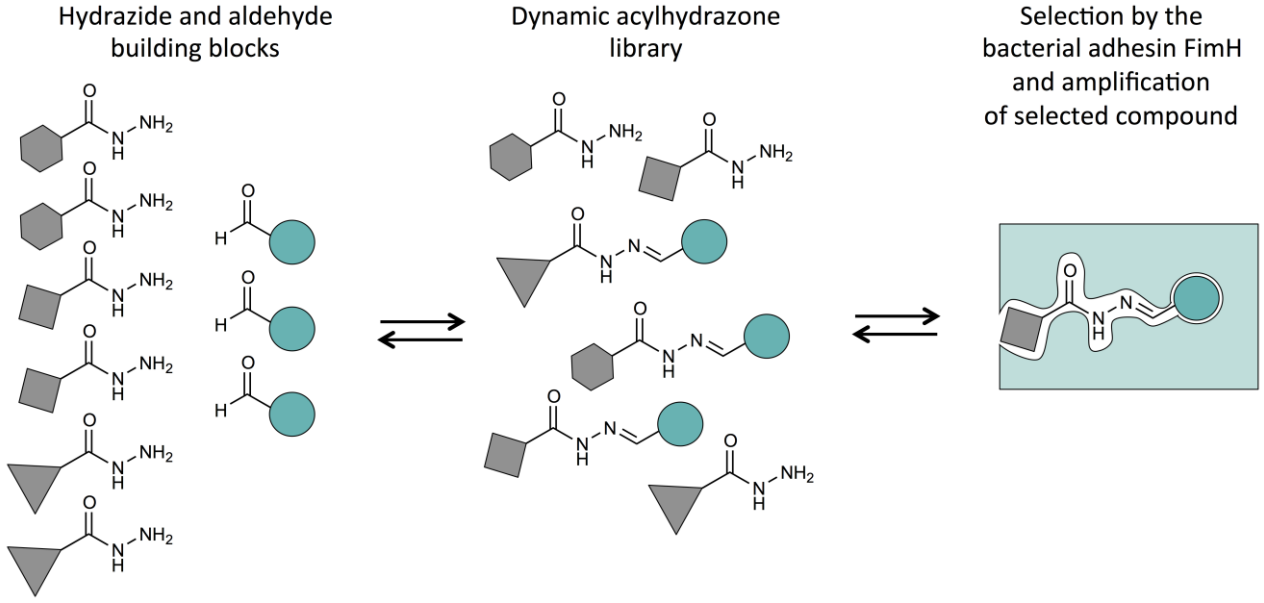


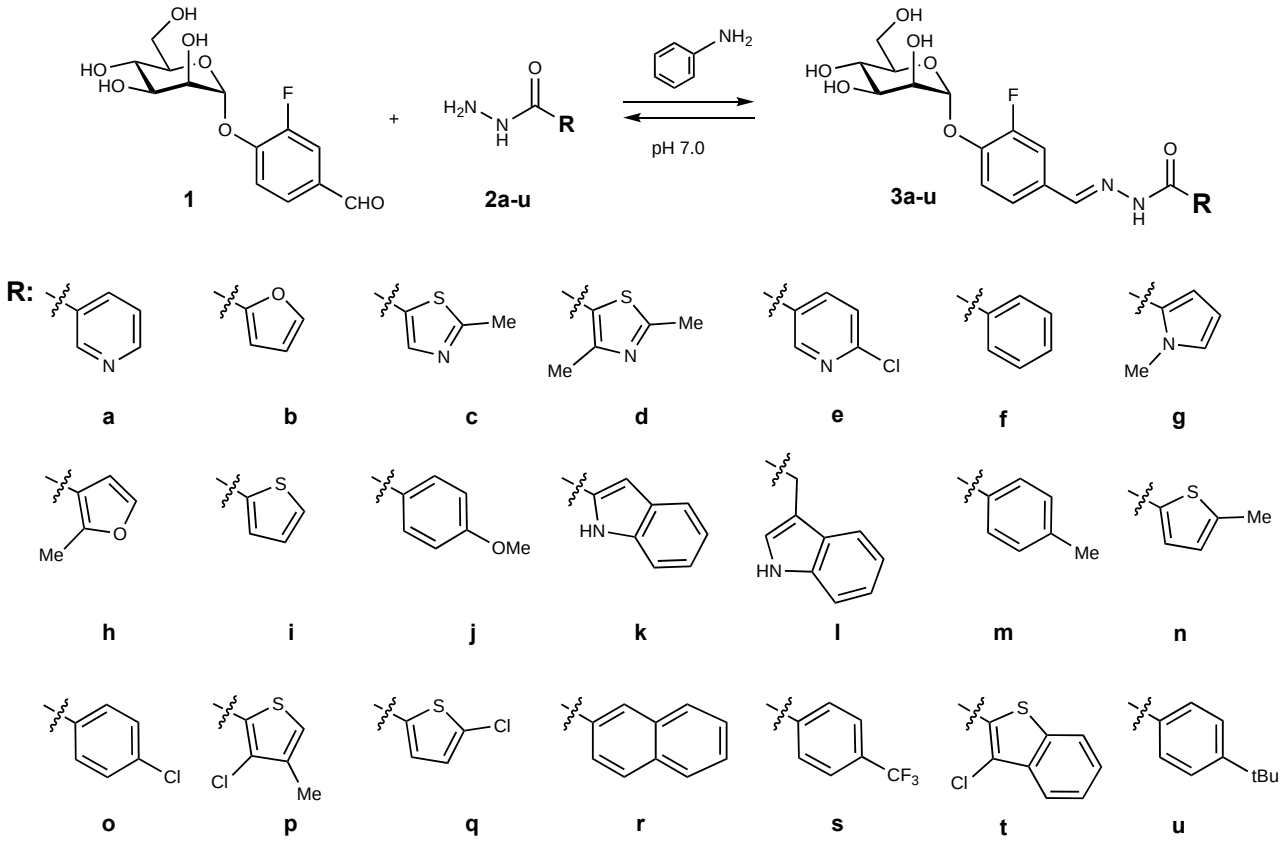
Figure 1. Schematic illustration of tdDCC. Reversibly reacting hydrazide and aldehyde building blocks generate a dynamic acylhydrazone library, which, when challenged with the target FimH, responds with a shift in its equilibrium composition including amplification of selected high-affinity ligands.

Whereas earlier reports focused on small libraries in a qualitative "hit/no-hit"-manner,[3-6] a more precise affinity ranking is required to increase the value of tdDCC. So far, the relationship between the extent of amplification and affinity was addressed in detail only by Nasr *et al*.[7] Furthermore, it has been noted that if several library constituents exhibit high affinity, the identification of the best binders might be difficult.[8] Finally, theoretical considerations regarding the relationship between host-ligand interactions and extent of amplification have been reported.[9]

In this communication, we have examined in greater detail the relationship between the amplification of library members in tdDCC experiments and their dissociation constants (K_D). As target protein we selected the bacterial adhesin FimH, located at the distal tip of type 1 pili of uropathogenic *E. coli* (UPEC) strains, which are the cause of the majority of urinary tract infections (UTI).[10-12] In the initial step of infection, FimH binds to the highly mannosylated surface protein uroplakin 1a on urothelial host cells,[13] a process which can be prevented with FimH antagonists such as aryl mannosides.[14-20]

We have reported previously that in addition to ligand affinity, also the ratio of scaffold to fragment building blocks, sample preparation, analysis, and method of data processing can be crucial factors in dictating experiment success.[21] Here, we extend the reported protocol for tdDCC using the bacterial adhesin FimH as

target protein to a larger scale. When aldehyde scaffold **1**[21] reacts reversibly with hydrazide fragments **2a**-**u** (→ **3a**-**u**; Scheme 1), the acylhydrazone library **3a**-**u** is obtained. At neutral pH in aqueous media, equilibrium formation is facilitated by aniline as a nucleophilic catalyst.[22]



Scheme 1. Aldehyde scaffold **1** and hydrazide fragments **2a**-**u** used for the reversible generation of acylhydrazones **3a**-**u** at neutral pH with aniline promotion.

Equilibration was carried out in the absence (blank library) or presence (template library) of biotinylated full-length FimH ($FimH_{FL-B}$)[23] for three days.[21] The reversibility of the acylhydrazone exchange reaction was then blocked by an adjustment in pH, which effectively locks in the library composition and renders the library suitable for analysis.[24] Prior to UV-HPLC analysis, the protein–ligand complex was captured using commercially available streptavidin agarose, and any unbound ligand removed from the sample (Figure 2A and 2B). The protein-bound ligand was then released by a further increase in pH together with addition of the FimH antagonist *n*-heptyl α-D-mannopyranoside[25] (**4**, Figure 2C). The supernatant was then analyzed with HPLC and the chromatograms of template libraries compared to those of equally treated blank samples.

On account of toxicological and stability concerns over the acylhydrazone moiety, we subsequently explored its potential for bioisosteric replacement.

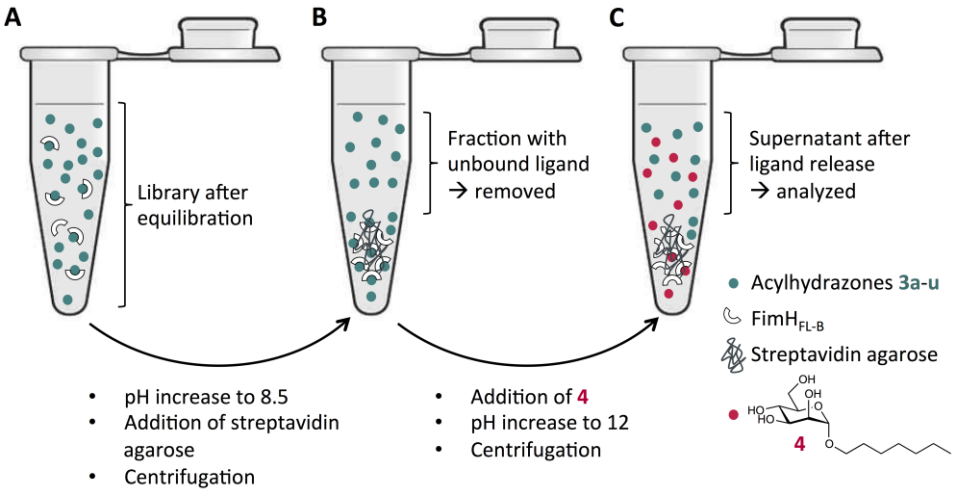


Figure 2. Schematic representation of the experimental tdDCC set-up.[21]

A. After sufficient equilibration, the library composition is made static through a pH increase (pH 8.5), and then the biotinylated target protein – including bound ligands – is captured with commercially available streptavidin agarose.
B. After centrifugation, the supernatant (containing unbound ligands) is discarded and the agarose-protein-ligand complex resuspended.
C. Bound ligands are released from the captured FimH into bulk solvent by the addition of excess of *n*-heptyl α-D-mannopyranoside (**4**) [25] and a further pH increase (pH 12). After a final centrifugation step, the supernatant is removed and analyzed by HPLC.

Results and Discussion

Target-directed dynamic combinatorial chemistry (tdDCC)

Because the outcomes of tdDCC experiments are influenced not only by the ratio of scaffolds to fragments,[21] but also by the ratio of library constituents to target protein,[26] we studied this issue in more detail by employing different amounts of building blocks, while keeping the protein concentration constant. Dynamic libraries were generated with varying concentrations of aldehyde scaffold **1** (600 μM, 400 μM, and 200 μM) together with hydrazide fragments **2a**-**u** each at 100 μM. All libraries were equilibrated in the presence and absence of 100 μM FimH$_{FL-B}$ (measured in triplicates). In this experimental design, the amount of scaffold **1** is determining the maximum attainable acylhydrazone concentration. Assuming full conversion of the aldehyde, facilitated by the addition of excess hydrazides, concentration ratios of 6:1, 4:1, and 2:1 between acylhydrazones and target protein should be reached. With a 1:1 ratio, accurate detection was not possible due to an insufficient amount of acylhydrazones, while unsatisfactory solubility became an issue at higher protein concentrations. The libraries were analyzed by UV-HPLC, detecting optical density at 310 nm. Conveniently, only acylhydrazones absorb UV light at this particular wavelength, but not other library constituents such as aniline, unreacted hydrazides, or *n*-heptyl α-D-mannopyranoside (**4**). Peaks in the resulting chromatograms were assigned using reference samples. Because both **3f** and **3g**, as well as **3m** and **3n**, gave overlapping signals which could not be resolved, we treated these signals as containing equal amounts of both constituents.

Composition of each library was determined based on the relative peak area (RPA), where the summated peak areas in each chromatogram were set to 100% and each peak assigned its fraction. Based on this information, the *normalized change of RPA*[21] between template and blank samples could be calculated and the influence of $FimH_{FL-B}$ on the library composition assessed (Figure 3). A positive value indicates amplification of a compound in the presence of $FimH_{FL-B}$, whereas a negative value indicates depletion.

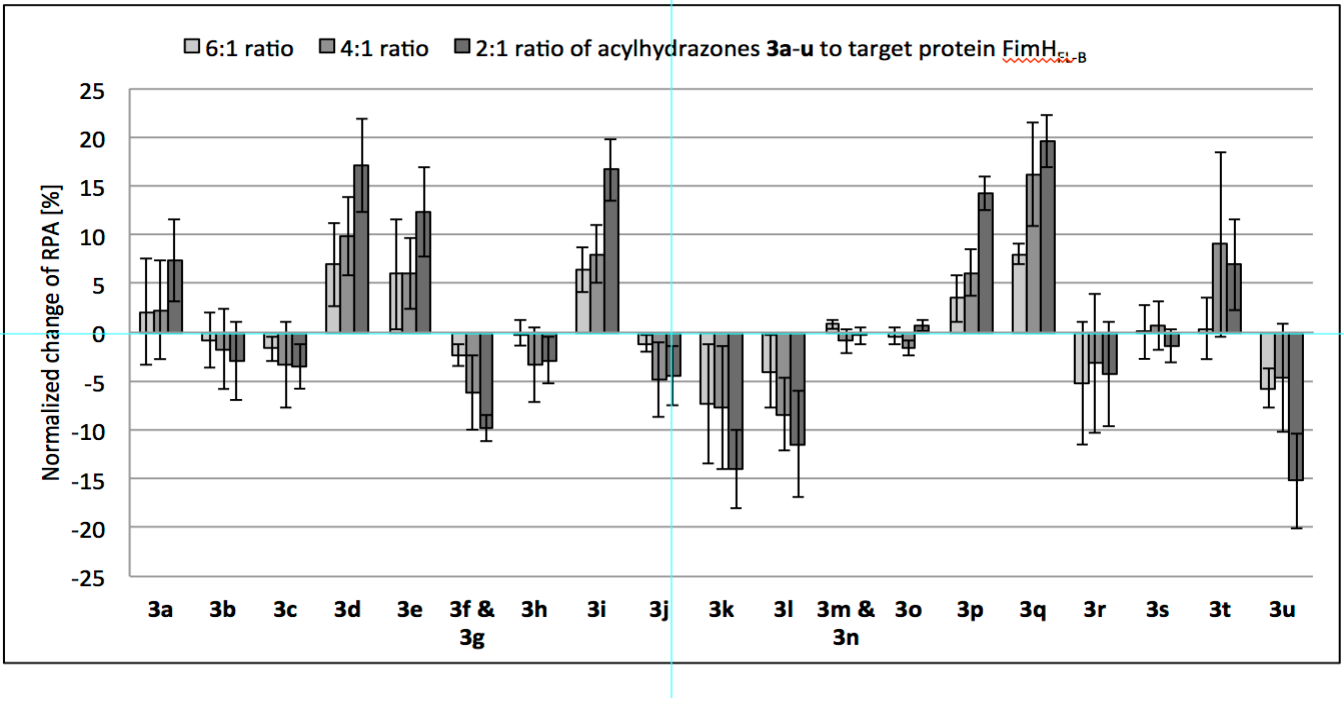


Figure 3. *Normalized change of RPA*[21] between template and blank libraries. Bars from light to dark grey indicate 6:1, 4:1, and 2:1 concentration ratios of total acylhydrazones to $FimH_{FL-B}$. Triplicates of all libraries were generated in the presence and absence of 100 μM $FimH_{FL-B}$. Error bars indicate error propagation of standard deviations of RPAs over three measurements.[21]

The most pronounced influence of $FimH_{FL-B}$ on the library composition was observed when core aldehyde **1** was present at 200 μM, resulting in a 2:1 ratio of acylhydrazones to target protein. When the scaffold was employed at higher concentrations (400 μM and 600 μM), changes in composition of the libraries were less pronounced. To evaluate if the *normalized changes of RPA* correlated with the affinities of the corresponding acylhydrazones, K_D values for **3a**-**u** were determined by surface plasmon resonance (SPR) using a previously established procedure.[21] Owing to the structural similarity of the compounds, differences in observed K_D values were not drastic (267 nM to 760 nM; Table 1). Obviously, this narrow distribution of affinities places highest demands on the applied analytical tools.

Table 1. Affinities measured by surface plasmon resonance

Compound	K_D [nM]	Compound	K_D [nM]
1	3160[21]	**3k**	427
3a	359	**3l**	508
3b	520[21]	**3m**	484
3c	358	**3n**	390
3d	267	**3o**	440
3e	492	**3p**	377
3f	550[21]	**3q**	286
3g	461	**3r**	337
3h	642	**3s**	536
3i	330[21]	**3t**	376
3j	462	**3u**	760[21]

Results of tdDCC experiments are commonly reported in a "hit/no-hit"-manner. Amplification ("hit") and depletion ("no-hit") were only considered significant when the propagated error did not cross the baseline (Figure 3). Otherwise, compound concentrations were regarded as unchanged. Ligands **3d**, **3i**, **3p** and **3q** were amplified by the target in each of the three described experimental designs, whereas **3a** and **3t** were only amplified in the replicates with a 2:1 acylhydrazones to $FimH_{FL-B}$ ratio. The investigation of compounds with a negative *normalized change of RPA* revealed that signals **3l**, **3u**, and overlapping **3f** and **3g** were decreased, in accordance with lower affinities. For acylhydrazones with intermediate affinities, the combined signal of **3m** and **3n** stayed unchanged with 200 μM and 400 μM, but was slightly increased with 600 μM of core aldehyde **1**. Finally, **3j** (K_D of 462 nM) and **3o** (K_D of 440 nM) remained unchanged in all libraries.

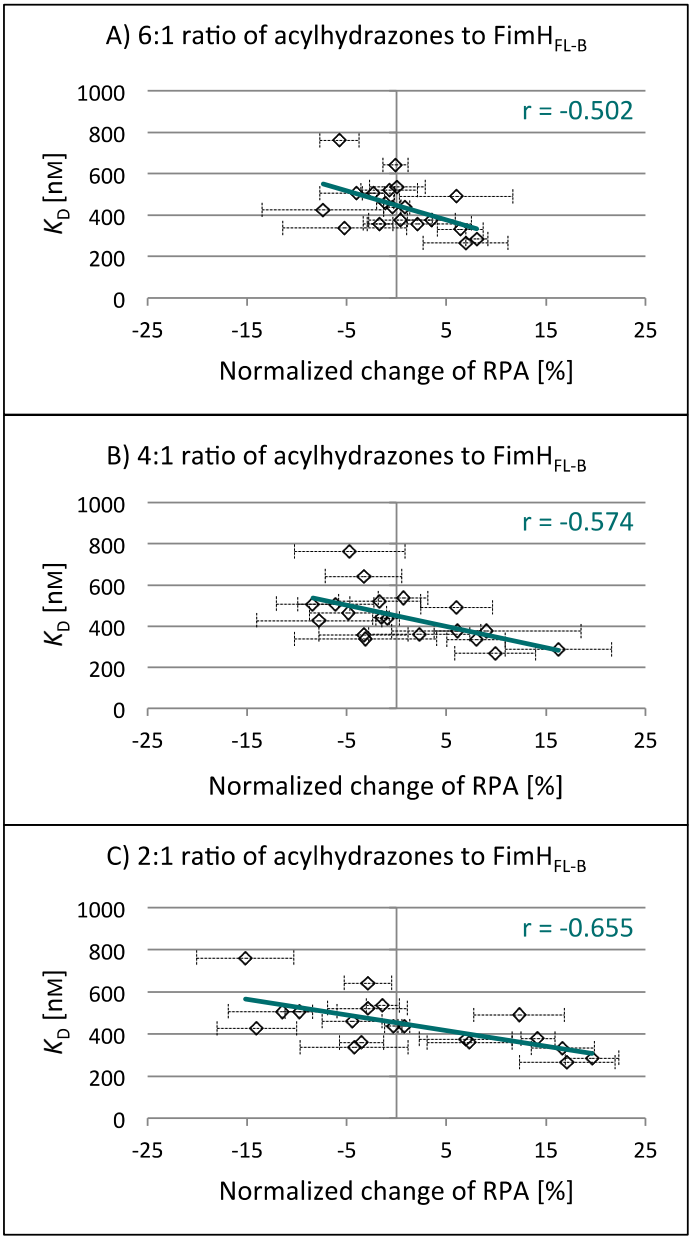


Figure 4. Quantitative relationship between the normalized change of relative peak area (RPA) and the dissociation constant (K_D) for different acylhydrazones. Pearson's correlation coefficient r is given as a measure of alignment.

Even though the majority of *normalized changes of RPA* correlated with the affinity data obtained by SPR, deviations were found for some acylhydrazones: albeit exhibiting rather high affinities of 358 nM and 337 nM, the *normalized changes of RPA* for **3c** and **3r** remained unchanged, while **3e** with a K_D of only 492 nM was amplified. Furthermore, **3b**, **3h**, and **3s** with rather low affinities of 523 nM, 642 nM, and 536 nM, respectively, remained unchanged, whereas **3k** with an affinity of 427 nM was decreased. Some of these aberrations could potentially result from the fast binding kinetics (both on- and off-rates) between acylhydrazones and FimH$_{FL-B}$, as was observed in the SPR experiments (see Supporting Information). Since the kinetic constants could not be uniquely determined in all cases, some of the reported K_D values may be erroneous. Apart from the fast binding kinetics[21] which may have impeded the SPR measurements, we currently have no explanation for these outliers. However, it is important to keep in mind that when different techniques for affinity

measurements are utilized, deviations resulting from some inherent errors of measurements are often unavoidable.

To assess the quantitative relationship between tdDCC and SPR results, the *normalized change of RPA* for each acylhydrazone was plotted against its K_D value. In the case of overlapping signals in the tdDCC experiment, the K_D value used was the average of the two acylhydrazones. Linear regression of the experimental data obtained using a 6:1 substrate ratio showed a moderate Pearson's correlation coefficient (r) of -0.502 (Figure 4A). For the 4:1 substrate ratio, the correlation coefficient slightly increased to -0.574 (Figure 4B), and for the 2:1 ratio, the highest correlation with an r-value of -0.655 was obtained (Figure 4C).

Overall, the tdDCC experiments delivered results comparable to SPR. Given the narrow range of K_D-values in the compound series, this clearly highlights the sensitivity of tdDCC. Furthermore, the tdDCC approach offers great economy of time: while SPR often requires independent synthesis and purification prior to affinity measurement, tdDCC combines the two steps into a single assay, thus clearly accelerating the process of hit identification. An additional benefit is that the described tdDCC protocol requires only standard laboratory equipment, while SPR requires an elaborate and costly instrument.

Bioisosteric replacement

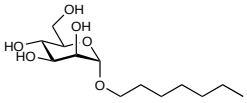
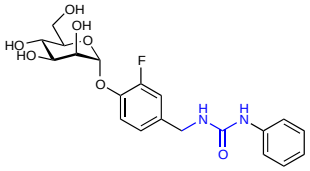
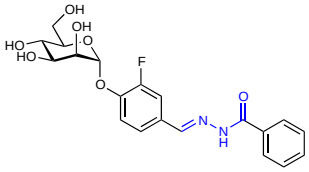
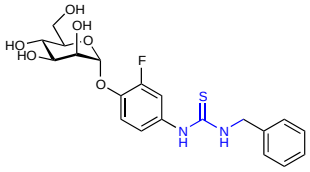
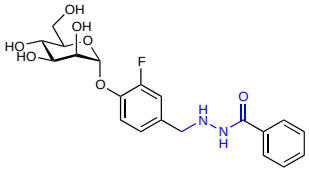
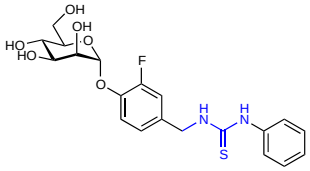
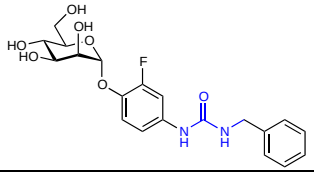
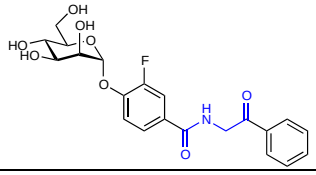
Besides their use in tdDCC,[21,24,27-31] several acylhydrazones have been reported to exhibit therapeutic properties[32] in areas such as cancer,[33-34] viral[35-36] and bacterial[37-38] infection, and pain and inflammation.[39] Furthermore, hydrazone linkages have been exploited for pH-responsive drug delivery.[40] However, both the cytotoxic activity inherently linked to anti-cancer drugs and the instability of the hydrazone moiety which affords its pH-responsiveness give rise to general concerns towards inclusion of the acylhydrazone group in potential FimH antagonists. Jumde *et al.* recently reported on bioisosteric replacement of the acylhydrazone moiety.[41] Therefore, in an effort to improve on stability and reduce toxicity, we generated a small library of bioisosteric analogues.

To explore possible bioisosteres of acylhydrazone, six alternatives to **3f** were synthesized (→ **5**-**10;** Table 2). Conveniently, reduction of **3f** with $NaBH_3CN$ yielded hydrazide **5**. Ureas **6** and **7** were synthesized from the corresponding anilines and amines, which were coupled via intermediates formed from 4-nitrophenyl chloroformate. Thioureas **8** and **9** were generated from the same aniline and amine starting materials through activation with 1,1'-thiocarbonyldiimidazole. Lastly, amide **10** was obtained by first assembling the aglycone from acid and amine precursors using standard peptide coupling, followed by mannosylation.

The affinities of compounds **5**-**10** were evaluated in a competitive fluorescence polarization assay (FPA),[14,17] using a non-biotinylated version of the FimH full-length protein ($FimH_{FL}$).[23] In type 1 pili of UPECs, the FimH subunit is stabilized by the N-terminal donor strand of the adjacent FimG subunit. Because isolated FimH turned out to be unstable, $FimH_{FL-B}$ and $FimH_{FL}$ required stabilization by a peptide consisting of the 15 terminal amino acids of FimG which mimics the donor strand. In the case of $FimH_{FL-B}$, biotin was linked to the pentadecapeptide, which does not alter $FimH_{FL-B}$'s binding properties as compared to $FimH_{FL}$. Hence, affinities determined with either of the constructs should be comparable. In the competitive FPA, the compounds under investigation displace a fluorescently labeled FimH antagonist (see **11** in the Supporting Information)[14] whose fluorescence polarization depends on target binding. By running a ligand dilution series, the dissociation constants could be determined and are summarized in Table 2. For **3f**, a K_D value of 515 nM was obtained, which is in excellent agreement with the affinity measured by SPR (550 nM; Table 1). All bioisosteres except for amide **10** exhibited a diminished affinity for $FimH_{FL}$. Noteworthy, only when the benzoyl moiety of the acylhydrazone was preserved as in hydrazide **5** and amide **10**, a strong loss of affinity could be avoided.

In summary, as evidenced by antagonist **10**, and as described by Jumde *et al.*[41] replacement by an amide provides a good starting point for further optimization of acylhydrazones.

Table 2. Dissociation constant K_D and relative K_D (rK_D) as determined with the fluorescece polarization assay

Cpd.	Structure	K_D [nM]	rK_D	Cpd.	Structure	K_D [nM]	rK_D
4		3600 [17]	-	**7**		3498	6.79
3f		515.0	1.00	**8**		2828	5.49
5		783.6	1.52	**9**		1673	3.24
6		1097	2.13	**10**		447.5	0.87

Conclusions

In tdDCC experiments, the resulting composition is generally only qualitatively ranked in a "hit/no-hit"-manner. One goal of this communication was to explore whether a tdDCC ranking correlated to an affinity ranking that had been determined using surface plasmon resonance (SPR) experiments. We therefore established a 21-membered acylhydrazone library using aldehyde scaffold **1** and the commercially available hydrazide fragments **2a**-**u** (→ **3a**-**u**). TdDCC acylhydrazone libraries were generated both in the absence and presence of biotinylated target protein $FimH_{FL-B}$, and then analyzed using UV-HPLC. By calculating the *normalized changes of relative peak area (RPA)* between template and blank libraries, the influence of $FimH_{FL-B}$ could be assessed. Surprisingly, the library composition observed post-equilibration was influenced by the acylhydrazones/$FimH_{FL-B}$ ratio (6:1, 4:1, and 2:1), where differences between components became more enhanced with greater relative amounts of $FimH_{FL-B}$ (*i.e.* up- or down-regulation was more pronounced). When the tdDCC results were qualitatively ranked in a "hit/no-hit"-manner, the majority of amplified acylhydrazones indeed also exhibited high affinities in SPR experiments, whereas lower K_Ds correlated with down-regulated compounds. Next, when the *normalized changes of RPA* were plotted against K_D values, a linear correlation was observed. The best alignment was obtained from the libraries with a 2:1 ratio of acylhydrazone to protein target, but the correlation was diminished when libraries with the 4:1 and 6:1 ratios were evaluated. These results suggest that a stoichiometric ratio between library constituents and target protein would be ideal for the generation of libraries in which all members exhibit affinity. However, different ratios are conceivable for

libraries, which cover a wider range of affinities. In a situation where only a few good binders are present, their formation would more efficiently outcompete the others.

In subsequent efforts, replacement of the potentially hazardous acylhydrazone moiety with various bioisosteres was investigated. Whereas urea (→ **6** and **7**) and thiourea (→ **8** and **9**) analogues of parent compound **3f** exhibited decreased affinities, hydrazide derivative **5** retained affinity and amide analogue **10** mildly enhanced the affinity, indicating that the latter two bioisosteres could represent a good starting point for further optimization of acylhydrazone hits from tdDCC.

In summary, applying tdDCC to $FimH_{FL-B}$ using acylhydrazone libraries of structurally related mannosides successfully confirmed the high sensitivity of this approach. Most importantly, a linear association between the *normalized change of RPA* and the K_D values determined by SPR could be observed.

Experimental Section

General. Affinity values were determined using a Biacore T200 system (GE Healthcare). UV-HPLC measurements were made using an Agilent 1100/1200 system (Agilent). FPA was measured on a Synergy H1 hybrid multimode microplate reader (BioTek Instruments Inc., Winooski, VT, USA).
FPA,[14,17] protein production,[21] SPR measurements,[21] and tdDCC experiments[21] were conducted as previously described. UV-HPLC analysis of libraries: Column: Waters Altlantis T3, 3 µm, 2.1 x 150 mm (Waters Corporation), solvent A: H_2O + 0.01% TFA (trifluoroacetic acid); solvent B: MeCN + 0.01% TFA. Detection: UV absorption at 310 nm. Gradient: 5% → 25% B (35 min) → 50% B (65 min), flow rate: 0.3 mL/min, injection volume: 20 µL.

Synthesis

General. NMR spectra were recorded on a Bruker Avance DMX-500 (500.1 MHz) spectrometer. Assignment of ^{1}H NMR and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC). Chemical shifts are expressed in ppm using residual $CHCl_3$, MeOH, or DMSO as references. Optical rotations were measured with a PerkinElmer Polarimeter 341 and infrared spectra were measured on a PerkinElmer Spectrum One FT-IR Spectrometer. Electrospray ionization mass spectrometry (ESI-MS) data were obtained on a Waters Micromass ZQ instrument. High resolution (HR)MS analysis were carried out using an Agilent 1100 LC equipped with a photodiode array detector and a Micromass QTOF I equipped with a 4 GHz digital-time converter. Reactions were monitored by thin layer chromatography (TLC) using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by UV light and/or by charring with a molybdate solution (0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Medium pressure liquid chromatography (MPLC) separations were carried out on a CombiFlash R_f (Teledyne ISCO, Inc.) with RediSep disposable normal-phase or RP-18 (LiChroprepRP18, Merck) reversed-phase flash columns. Commercially available reagents were purchased from Fluka, Aldrich, Alfa Aesar, Fluorochem, and Apollo. Solvents were purchased from Sigma-Aldrich, Acros Organics, or VWR.

Synthesis of the acylhydrazone library

General procedure A for acylhydrazone formation. A flask was charged with a magnetic stirrer, then aldehyde **1**[21] and hydrazide **2a,c-e,g-h,j-t** were dissolved in H_2O/MeCN (2 mL, 7:3). AcOH (100 µL) was added and the mixture was stirred at r.t. for 5-22 h until only product was detected by MS. Then, the mixture was neutralized with 1 M aq. NaOH and the solvents were removed under reduced pressure. The residue was purified by MPLC

on RP-18 (H_2O/MeCN, 5:95 to 20:80) to give the desired products **3a,c-e,g-h,j-t**. For synthesis of **3b**, **3f**, **3i** and **3u**, please see reference 21.

Note: Compounds **3a**, **3d**, and **3l** were obtained as inseparable mixtures of *E*- and *Z*-isomers, with the *E*-isomer most likely representing the bigger fraction due to its sterically more favorable conformation. For further evaluation, we conducted a high temperature NMR measurement (CD_3OD, 60 °C) with compound **3l**, which clearly showed a decreased resolution, suggesting faster conversion of the two isomers. Further, HPLC traces at concentrations similar to the DCC experiments showed only one peak. When the reaction was catalyzed by aniline instead of AcOH, the same *E*/*Z*-ratio was obtained. Thus, the ratio of isomers is expected to be similar in tdDCC and SPR experiments.

***N'*-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]nicotinohydrazide** (**3a**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and nicotinic hydrazide (**2a**, 4.5 mg, 33.1 µmol). Yield: 8.1 mg (58%) as an inseparable mixture of *E*- and *Z*-isomers (approx. 5:1). NMR data are given for the *E*-conformer. $[\alpha]_D^{20}$ +129.0 (*c* 0.35, MeOH); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ = 3.39–3.54 (m, 4H, H-4, H-5, H-6a, H-6b), 3.60 (dd, *J* 3.8, 10.6 Hz, 1H), 3.69 (d, *J* 7.8 Hz, 1H, H-3), 3.88 (s, 1H, H-2), 4.47 (s, 1H, OH-4), 4.89 (m, 2H, OH-3, OH-6), 5.14 (s, 1H, OH-2), 5.50 (s, 1H, H-1), 7.45 (t, *J* 8.3 Hz, 1H, Ar-H), 7.50 (d, *J* 8.6 Hz, 1H, Ar-H), 7.52–7.58 (m, 1H, Ar-H), 7.61 (d, *J* 11.9 Hz, 1H, Ar-H), 8.25 (d, *J* 7.7 Hz, 1H, Ar-H), 8.38 (s, 1H, *H*C=N), 8.75 (d, *J* 4.3 Hz, 1H, Ar-H), 9.06 (s, 1H, Ar-H), 12.05 (s, 1H, NH); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 60.9 (C-6), 66.5 (C-4), 69.8 (C-2), 70.5 (C-3), 75.5 (C-5), 99.7 (C-1), 113.9 (d, $J_{C,F}$ 20 Hz), 118.6, 123.6, 124.3, 129.1 (d, $J_{C,F}$ 6 Hz), 129.4, 135.4, 145.4 (d, $J_{C,F}$ 11 Hz; 8C, Ar-C), 146.9 (d, $J_{C,F}$ 2 Hz, H*C*=N), 148.6, 152.1 (2C, Ar-C), 152.5 (d, $J_{C,F}$ 245 Hz, Ar-C), 161.8 (C=O); IR (KBr): ν 3429 (vs, OH, NH), 1652 (vs, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{19}H_{20}FN_3NaO_7$ $[M+Na]^+$: 444.1183, found: 444.1181.

(*E*)-*N'*-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-2-methylthiazole-5-carbohydrazide (**3c**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 2-methyl-thiazole-4-carboxylic acid hydrazide (**2c**, 5.2 mg, 33.1 µmol). Yield: 8.4 mg (57%). $[\alpha]_D^{20}$ +117.7 (*c* 0.24, MeOH); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 2.75 (s, 3H, CH_3), 3.41 (m, 1H, H-5), 3.44–3.54 (m, 2H, H-6a, H-4), 3.60 (dd, *J* 5.7, 11.2 Hz, 1H, H-6b), 3.88 (s, 1H, H-3), 3.69 (s, 1H, H-2), 4.46 (t, *J* 5.7 Hz, 1H, OH-6), 4.82 (d, *J* 5.4 Hz, 1H, OH-3), 4.87 (d, *J* 5.5 Hz, 1H, OH-4), 5.11 (d, *J* 3.3 Hz, 1H, OH-2), 5.49 (s, 1H, H-1), 7.38–7.49 (m, 2H, Ar-H), 7.55 (d, *J* 11.7 Hz, 1H, Ar-H), 8.29 (s, 1H, Ar-H), 8.51 (s, 1H, *H*C=N), 11.79 (s, 1H, NH); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 18.8 (CH_3), 60.9 (C-6), 66.5 (C-4), 69.8 (C-2), 70.6 (C-3), 75.5 (C-5), 99.7 (C-1), 113.7 (d, $J_{C,F}$ 20 Hz), 118.6, 124.4 (d, $J_{C,F}$ 2 Hz), 125.4, 129.3 (d, *J* 7 Hz), 145.3 (d, $J_{C,F}$ 11 Hz; 6C, Ar-C), 147.1 (H*C*=N), 148.3, 152.5 (d, $J_{C,F}$ 245 Hz), 156.9 (3C, Ar-C), 166.5 (C=O); IR (KBr): ν 3413 (vs, OH, NH), 1659 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{18}H_{20}FN_3NaO_7S$ $[M+Na]^+$: 464.0904, found: 464.0905.

***N'*-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-2,4-dimethylthiazole-5-carbohydrazide** (**3d**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 2,4-dimethyl-thiazole-5-carboxylic acid hydrazide (**2d**, 5.7 mg, 33.1 µmol). Yield: 3.2 mg (21%) as a 5:1 mixture of *E*- and *Z*-conformer. NMR data are given for the *E*-conformer. $[\alpha]_D^{20}$ +110.5 (*c* 0.11, MeOH); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 2.51, 2.66 (2 s, 6H, 2 CH_3), 3.41 (d, *J* 7.8 Hz, 1H, H-5), 3.43–3.55 (m, 2H, H-4, H-6a), 3.60 (dd, *J* 5.3, 11.1 Hz, 1H, H-6b), 3.68 (d, *J* 8.4 Hz, 1H, H-3), 3.88 (s, 1H, H-2), 4.46 (t, *J* 5.7 Hz, 1H, OH-6), 4.85 (s, 1H, OH-3), 4.88 (d, *J* 12.0 Hz, 1H, OH-4), 5.13 (s, 1H, OH-2), 5.49 (s, 1H, H-1), 7.45 (m, 1H, Ar-H), 7.50 (d, *J* 7.2 Hz, 1H, Ar-H), 7.57 (d, *J* 11.7 Hz, 1H, Ar-H), 8.02 (s, 1H, *H*C=N), 11.72 (s, 1H, NH); ^{13}C NMR (125 MHz, $(CD_3)_2SO$):δ 18.5 (2C, 2 *C*H_3), 60.9 (C-6), 66.5 (C-4), 69.8 (C-2), 70.6 (C-3), 75.5 (C-5), 99.7 (C-1), 114.2 (d, $J_{C,F}$ 19 Hz), 118.8, 124.2 (d, $J_{C,F}$ 3 Hz), 128.9, 142.3, 145.2 (d, $J_{C,F}$ 11 Hz; 6C, Ar-C), 150.5 (H*C*=N), 152.4 (d, $J_{C,F}$ 245 Hz), 160.3, 162.2 (3C, Ar-C), 170.2 (C=O); IR (KBr): ν 3436 (OH, NH, vs), 1646 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{19}H_{22}FN_3NaO_7S$ $[M+Na]^+$: 478.1060, found: 478.1061.

(*E*)-6-Chloro-*N*'-[3-fluoro-4-(α-D-mannopyranosyloxy)benzylidene]nicotinohydrazide (**3e**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 6-chloropyridine-3-carbohydrazide (**2e**, 5.7 mg, 33.1 µmol). Yield: 3.8 mg (25%). $[\alpha]_D^{20}$ +74.1 (*c* 0.30, MeOH); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 3.38–3.60 (m, 4H, H-6a, H-6b, H-5, H-4), 3.68 (d, *J* 9.1 Hz, 1H, H-3), 3.88 (s, 1H, H-2), 5.47 (s, 1H, H-1), 7.41 (m, 1H, Ar-H), 7.45 (d, *J* 8.6 Hz, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.62 (d, *J* 3.0 Hz, 1H, Ar-H), 8.35 (d, *J* 8.3 Hz, 1H, *H*C=N), 8.37 (d, *J* 9.6 Hz, 1H, Ar-H), 8.93 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 60.9 (C-6), 66.4 (C-4), 69.8 (C-2), 70.5 (C-3), 75.5 (C-5), 99.8 (C-1), 113.7 (d, $J_{C,F}$ 20 Hz), 118.50, 123.8, 124.1, 138.9 (8C, Ar-C), 147.0 (H*C*=N), 149.3, 151.9, 152.4 (d, $J_{C,F}$ 244 Hz; 3C, Ar-C), 174.0 (C=O); IR (KBr): ν 3436 (vs, OH, NH), 1634 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{19}H_{19}ClFN_3NaO_7$ $[M+Na]^+$: 478.0793, found: 478.0799.

(*E*)-*N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-1-methyl-1*H*-pyrrole-2-carbohydrazide (**3g**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 1-methyl-1*H*-pyrrole-2-carboxylic acid hydrazide (**2g**, 4.6 mg, 33.1 µmol). Yield: 9.5 mg (68%). $[\alpha]_D^{20}$ +132.5 (*c* 0.61, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.65 (m, 1H, H-5), 3.69–3.81 (m, 3H, H-4, H-6a, H-6b), 3.93 (dd, *J* 3.1, 9.6 Hz, 1H, H-3), 3.96 (s, 3H, CH_3), 4.08 (s, 1H, H-2), 5.56 (s, 1H, H-1), 6.13 (s, 1H, Ar-H), 6.93 (s, 2H, Ar-H), 7.41 (t, *J* 8.1 Hz, 1H, Ar-H), 7.45 (d, *J* 8.6 Hz, 1H, Ar-H), 7.71 (d, *J* 11.9 Hz, 1H, Ar-H), 8.15 (s, 1H, *H*C=N); ^{13}C NMR (125 MHz, CD_3OD): δ 37.0 (CH_3), 62.6 (C-6), 68.2 (C-4), 71.8 (C-2), 72.3 (C-3), 76.0 (C-4), 101.2 (C-1), 108.7, 115.2 (d, $J_{C,F}$ 20 Hz), 119.5, 124.8, 125.7 (d, *J* 4 Hz), 130.7, 131.4 (d, $J_{C,F}$ 7 Hz; 7C, Ar-C), 146.7 (d, $J_{C,F}$ 6 Hz, H*C*=N), 147.0 (d, $J_{C,F}$ 11 Hz, Ar-C), 152.4 (C=O), 154.6 (d, $J_{C,F}$ 246 Hz, Ar-C); IR (KBr): ν 3436 (vs, OH, NH), 1634 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{19}H_{22}FN_3NaO_7$ $[M+Na]^+$: 446.1339, found: 446.1341.

(*E*)-*N*'-(3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene)-2-methylfuran-3-carbohydrazide (**3h**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 2-methyl-furan-carboxylic acid hydrazide (**2h**, 4.6 mg, 33.1 µmol). Yield: 9.1 mg (65%). $[\alpha]_D^{20}$ +98.9 (*c* 0.56, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 2.63 (s, 3H, CH_3), 3.67 (d, *J* 3.6 Hz, 1H, H-5), 3.72–3.83 (m, 3H, H-4, H-6a, H-6b), 3.95 (dd, *J* 2.8, 9.4 Hz, 1H, H-3), 4.10 (s, 1H, H-2), 5.59 (s, 1H, H-1), 6.85 (s, 1H, Ar-H), 7.42–7.52 (m, 3H, Ar-H), 7.76 (d, *J* 11.9 Hz, 1H, Ar-H), 8.22 (s, 1H, *H*C=N); ^{13}C NMR (125 MHz, CD_3OD): δ 13.7 (CH_3), 62.6 (C-6), 68.2 (C-4), 71.7 (C-2), 72.3 (C-3), 76.0 (C-5), 101.2 (C-1), 109.4, 115.0, 115.3 (d, $J_{C,F}$ = 20 Hz), 119.4, 126.0, 142.1, 147.3 (d, $J_{C,F}$ 11 Hz; 8C, Ar-C), 147.9 (d, $J_{C,F}$ 1 Hz, H*C*=N), 154.6 (d, $J_{C,F}$ 246 Hz), 160.0 (2C, Ar-C), 163.1 (C=O); IR (KBr): ν 3414 (vs, OH, NH), 1619 (vs, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{19}H_{21}FN_2Na\ O_8$ $[M+Na]^+$: 447.1182, found: 447.1182.

(*E*)-*N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-4-methoxybenzhydrazide (**3j**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 4-methoxybenzhydrazide (**2j**, 5.5 mg, 33.1 µmol). Yield: 4.0 mg (27%). $[\alpha]_D^{20}$ +106.6 (*c* 0.26, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.64 (m, 1H, H-5), 3.69–3.81 (m, 3H, H-4, H-6a, H-6b), 3.88 (s, 3H, CH_3), 3.93 (dd, *J* 3.0, 9.4 Hz, 1H, H-3), 4.08 (s, 1H, H-2), 5.57 (s, 1H, H-1), 7.05 (d, *J* 8.5 Hz, 2H, Ar-H), 7.43 (t, *J* 8.2 Hz, 1H, Ar-H), 7.49 (d, *J* 8.5 Hz, 1H, Ar-H), 7.77 (d, *J* 11.9 Hz, 1H, Ar-H), 7.92 (d, *J* 8.6 Hz, 2H, Ar-H), 8.25 (s, 1H, *H*C=N); ^{13}C NMR (125 MHz, CD_3OD): δ 56.0 (CH_3), 62.6 (C-6), 68.2 (C-4), 71.7 (C-2), 72.3 (C-3), 76.0 (C-5), 101.2 (C-1), 115.0 (2C), 115.4 (d, $J_{C,F}$ 20 Hz), 119.5, 125.96, 126.07 (d, $J_{C,F}$ 2 Hz; 6C, Ar-C), 130.7 (2C), 131.1 (d, $J_{C,F}$ 7 Hz), 147.3 (d, $J_{C,F}$ 10 Hz; 4C, Ar-C), 148.4 (H*C*=N), 154.6 (d, $J_{C,F}$ 246 Hz, Ar-C), 164.5 (C=O), 166.7 (Ar-C); IR (KBr): ν 3439 (vs, OH, NH), 1648 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{21}H_{23}FN_2NaO_8$ $[M+Na]^+$: 473.1336, found: 473.1336.

(*E*)-*N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-1*H*-indole-2-carbohydrazide (**3k**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 1*H*-indole-2-carboxylic acid hydrazide (**2k**, 5.8 mg, 33.1 µmol). Yield: 7.7 mg (51%). $[\alpha]_D^{20}$ +100.9 (*c* 0.47,MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.65 (m, 1H, H-5), 3.70–3.82 (m, 3H, H-4, H-6a, H-6b), 3.93 (dd, *J* 3.0, 9.4 Hz, 1H, H-3), 4.09 (s, 1H, H-2), 5.56 (s, 1H, H-1), 7.15–7.27 (m, 2H, Ar-H), 7.38–7.50 (m, 3H, Ar-H), 7.73 (s, 1H, Ar-H), 8.06 (d, *J* 6.4 Hz, 1H,

Ar-H), 8.17 (s, 1H, *H*C=N), 8.22 (d, *J* 7.1 Hz, 1H, Ar-H); ^{13}C NMR (125 MHz, CD_3OD): δ 62.6 (C-6), 68.2 (C-4), 71.8 (C-2), 72.3 (C-3), 76.0 (C-5), 101.2 (C-1), 112.8, 115.2 (d, $J_{C,F}$ 19 Hz), 119.5, 122.25, 122.31, 123.9, 125.7, 127.6, 127.7, 129.6, 131.5 (d, $J_{C,F}$ 7 Hz), 138.0 (12C, Ar-C), 146.2 (H*C*=N), 147.0 (d, $J_{C,F}$ 11 Hz), 154.6 (d, $J_{C,F}$ 246 Hz; 2C, Ar-C), 157.3 (C=O); IR (KBr): ν 3415 (vs, OH, NH), 1619 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{22}H_{22}FN_3NaO_7$ $[M+Na]^+$: 482.1339, found: 482.1340.

***N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-2-(1*H*-indol-2-yl)acetohydrazide (3l).** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and indole-3-acetic hydrazide (**2l**, 6.3 mg, 33.1 µmol). Yield: 12.8 mg (82%) as a 2:1 mixture of *E*- and *Z*-conformation. $[\alpha]_D^{20}$ +99.1 (*c* 0.39, MeOH); ^{1}H NMR (500 MHz, CD_3OD; *E*:*Z* = 2:1, normalized to *E* conformation): δ 3.61–3.68 (m, 1.5H, H-5 *E*+*Z*), 3.70–3.81 (m, 6.5H, CH_2 *E*, H-6a *E*+*Z*, H-6b *E*+*Z*, H-4 *E*+*Z*), 3.91–3.96 (m, 1.5H, H-3 *E*+*Z*), 4.08 (dd, *J* 1.9, 3.3 Hz, 1H, H-2 *E*), 4.10 (dd, *J* 1.9, 3.4 Hz, 0.5H, H-3 *Z*), 4.20 (s, 1H, CH_2 *Z*), 5.57 (d, *J* 1.6 Hz, 1H, H-1 *E*), 5.58 (d, *J* 1.6 Hz, 0.5H, H-1 *Z*), 6.99–7.07 (m, 2H, Ar-H *E*+*Z*), 7.08–7.15 (m, 1.5H, Ar-H *E*+*Z*), 7.21 (s, 0.5H, Ar-H *Z*), 7.25 (s, 1H, Ar-H *E*), 7.47–7.33 (m, 4.5H, 3 Ar-H *E*, 3 Ar-H *Z*), 7.57 (d, *J* 12.6 Hz, 0.5H, Ar-H *Z*), 7.61 (d, *J* 7.9 Hz, 1H, Ar-H *E*), 7.65 (d, *J* 8.0 Hz, 0.5H, Ar-H *Z*), 7.70 (dd, *J* 1.6, 12.0 Hz, 1H, Ar-H *E*), 7.91 (s, 0.5H, CH=N *Z*), 8.06 (s, 1H, CH=N *E*); ^{13}C NMR (125 MHz, CD_3OD; only *E* conformer): δ 32.7 (CH_2), 62.6 (C-6), 68.2 (C-4), 71.8 (C-2), 72.3 (C-3), 76.0 (C-5), 101.2 (C-1), 126.1, 124.9, 122.6, 122.4, 120.0, 119.4, 115.5, 112.4, 101.4, 148.1 (16 C, 14 Ar-C, H*C*=N, C=O); IR (KBr): ν 3429 (vs, OH, NH), 1651 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{23}H_{24}FN_3NaO_7$ $[M+Na]^+$: 496.1496 found: 496.1496.

(*E*)-*N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-4-methylbenzohydrazide (3m). Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and *p*-toluic hydrazide (**2m**, 5.0 mg, 33.1 µmol). Yield: 7.7 mg (54%). $[\alpha]_D^{20}$ +104.2 (*c* 0.47, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 2.42 (s, 3H, CH_3), 3.65 (d, *J* 5.4 Hz, 1H, H-5), 3.70–3.81 (m, 3H, H-4, H-6a, H-6b), 3.93 (d, *J* 9.3 Hz, 1H, H-3), 4.08 (s, 1H, H-2), 5.57 (s, 1H, H-1), 7.34 (d, *J* 7.8 Hz, 2H, Ar-H), 7.43 (t, *J* 8.2 Hz, 1H, Ar-H), 7.49 (d, *J* 8.5 Hz, 1H, Ar-H), 7.76 (d, *J* 11.9 Hz, 1H, Ar-H), 7.83 (d, *J* 7.7 Hz, 2H, Ar-H), 8.25 (s, 1H, *H*C=N); ^{13}C NMR (125 MHz, CD_3OD): δ 21.5 (CH_3), 62.6 (C-6), 68.2 (C-4), 71.7 (C-2), 72.3 (C-3), 76.0 (C-5), 101.2 (C-1), 115.4 (d, $J_{C,F}$ 21 Hz), 119.4, 126.1, 128.8 (2C), 130.4 (2C), 131.0 (d, $J_{C,F}$ 7 Hz), 131.2, 144.3, 147.4 (d, $J_{C,F}$ 12 Hz; 11C, Ar-C), 148.8 (H*C*=N), 154.6 (d, $J_{C,F}$ 246 Hz, Ar-C), 167.1 (C=O); IR (KBr): ν 3421 (vs, OH, NH), 1651 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{21}H_{23}FN_2NaO_7$ $[M+Na]^+$: 457.1387, found: 457.1387.

(*E*)-*N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-5-methylthiophene-2-carbohydrazide (3n). Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 5-methyl-thiophene-2-carboxylic acid hydrazide (**2n**, 5.2 mg, 33.1 µmol). Yield: 7.8 mg (54%). $[\alpha]_D^{20}$ +156.0 (*c* 0.47, MeOH); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 2.52 (s, 3H, CH_3), 3.39–3.55 (m, 3H, H-4, H-5, H-6a), 3.60 (dd, *J* 4.9, 10.9 Hz, 1H, H-6b), 3.69 (d, *J* 8.9 Hz, 1H, H-3), 3.88 (s, 1H, H-2), 4.46 (t, *J* 5.8 Hz, 1H, OH-6), 4.83 (d, *J* 3.6 Hz, 1H, OH-3), 4.87 (t, *J* 8.4 Hz, 1H, OH-4), 5.12 (d, *J* 3.0 Hz, 1H, OH-2), 5.50 (s, 1H, H-1), 6.92 (s, 1H, Ar-H), 7.39–7.66 (m, 3H, Ar-H), 7.78 (m, 1H, Ar-H), 8.18 (s, 1H, *H*C=N), 11.75 (s, 1H, NH); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 15.1 (CH_3), 60.9 (C-6), 66.5 (C-5), 69.9 (C-2), 70.6 (C-3), 75.5 (C-4), 99.7 (C-1), 114.03 (d, $J_{C,F}$ 38 Hz), 118.8, 124.2, 125.4, 129.2, 135.1, 142.5, 145.49 (d, $J_{C,F}$ 79 Hz), 152.5 (d, $J_{C,F}$ 245 Hz), 161.2 (11C, 10 Ar-C, H*C*=N), 166.4 (C=O); IR (KBr): ν 3413 (vs, OH, NH), 1619 (vs, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{19}H_{21}FN_2NaO_7S$ $[M+Na]^+$: 463.0951, found: 463.0954.

(*E*)-4-Chloro-*N*'-[3-fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-carbohydrazide (3o). Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 4-chlorobenzhydrazide (**2o**, 5.6 mg, 33.1 µmol). Yield: 4.5 mg (30%). $[\alpha]_D^{20}$ +112.4 (*c* 0.24, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 3.63 (ddd, *J* 2.2, 5.3, 9.5 Hz, 1H, H-5), 3.69–3.82 (m, 3H, H-4, H6a, H-6b), 3.93 (dd, *J* 3.4, 9.4 Hz, 1H, H-3), 4.08 (dd, *J* 1.7, 3.1 Hz, 1H, H-2), 5.57 (s, 1H, H-1), 7.44 (t, *J* 8.3 Hz, 1H, Ar-H), 7.50 (d, *J* 9.2 Hz, 1H, Ar-H), 7.54 (d, *J* 8.5 Hz, 2H, Ar-H), 7.76

(m, 1H, Ar-H), 7.92 (d, *J* 8.5 Hz, 2H, Ar-H), 8.26 (s, 1H, *H*C=N); ^{13}C NMR (125 MHz, CD_3OD): δ 62.6 (C-6), 68.2 (C-4), 71.7 (C-2), 72.3 (C-3), 76.0 (C-5), 101.1 (C-1), 115.5 (d, $J_{C,F}$ 20 Hz), 119.4, 126.2, 130.0 (2C), 130.5 (2C), 130.8 (d, $J_{C,F}$ 7 Hz), 132.8, 139.5, 147.5 (d, $J_{C,F}$ 11 Hz; 11C, Ar-C), 149.3 (H*C*=N), 154.6 (d, $J_{C,F}$ 246 Hz, Ar-C), 165.9 (C=O); IR (KBr): ν 3436 (vs, OH, NH), 1651 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{20}H_{20}ClFN_2NaO_7$ $[M+Na]^+$: 477.0841, found: 477.0841.

(*E*)-3-Chloro-*N*'-[(3-fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-4-methylthiophene-2-carbohydrazide (**3p**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 3-chloro-4-methyl-2-thiophenecarboxylic acid hydrazide (**2p**, 6.3 mg, 33.1 µmol). Yield: 5.4 mg (34%). $[\alpha]_D^{20}$ +90.7 (*c* 0.15, MeOH); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 2.20 (s, 3H, CH_3), 3.37–3.55 (m, 3H, H-6a, H-4, H-5), 3.60 (dd, *J* 3.6, 11.2 Hz, 1H, H-6b), 3.68 (dd, *J* 2.9, 9.0 Hz, 1H, H-3), 3.87 (s, 1H, H-2), 4.47 (s, 1H, OH-6), 4.90 (s, 2H, OH-3, OH-4), 5.13 (s, 1H, OH-2), 5.49 (s, 1H, H-1), 7.40–7.48 (m, 2H, Ar-H), 7.56 (d, *J* 11.9 Hz, 1H, Ar-H), 7.65 (m, 1H, Ar-H), 8.06 (m, 1H, Ar-H), 11.77 (s, 1H, NH); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 14.3 (CH_3), 60.9 (C-6), 66.5 (C-4), 69.8 (C-2), 70.5 (C-3), 75.5 (C-5), 99.7 (C-1), 113.9 (d, $J_{C,F}$ 20 Hz), 118.6 (d, $J_{C,F}$ 6 Hz), 124.3, 129.1, 131.2, 136.6, 143.6, 143.8 (d, $J_{C,F}$ 9 Hz), 145.3 (9C, Ar-C), 146.8 (H*C*=N), 152.4 (d, $J_{C,F}$ = 245 Hz, Ar-C), 171.2 (C=O); IR (KBr): ν 3430 (vs, OH, NH), 1642 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{19}H_{20}ClFN_2NaO_7S$ $[M+Na]^+$: 497.0561, found: 497.0561.

(*E*)-5-Chloro-*N*'-[3-fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-thiophene-2-carbohydrazide (**3q**). Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 5-chlorothiophene-2-carboxylic acid hydrazide (**2q**, 5.6 mg, 33.1 µmol). Yield: 3.9 mg (26%). $[\alpha]_D^{20}$ +138.3 (*c* 0.15, MeOH); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 3.40–3.56 (m, 3H, H-4, H-5, H-6a), 3.62 (dd, *J* 4.1, 11.2 Hz, 1H, H-6b), 3.70 (dd, *J* 3.0, 9.1 Hz, 1H, H-3), 3.90 (s, 1H, H-2), 4.49 (t, *J* 5.6 Hz, 1H, OH-4), 4.89 (m, 2H, OH-3, OH-6), 5.15 (s, 1H, OH-2), 5.53 (s, 1H, H-1), 7.29 (d, *J* 4.1 Hz, 1H, Ar-H), 7.43–7.69 (m, 3H, Ar-H), 8.39 (m, 2H, Ar-H, *H*C=N), 12.00 (s, 1H, NH); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 61.0 (C-6), 66.5 (C-5), 69.8 (C-2), 70.5 (C-3), 75.5 (C-4), 99.7 (C-1), 114.4 (d, $J_{C,F}$ 19 Hz), 118.8, 124.4, 126.6, 128.6 (d, $J_{C,F}$ 6 Hz), 130.4, 134.5, 137.3 (8C, Ar-C), 143.5 (H*C*=N), 145.4 (d, $J_{C,F}$ 11 Hz), 152.4 (d, $J_{C,F}$ 245 Hz; 2C, Ar-C), 160.0 (C=O); IR (KBr): ν 3436 (vs, OH, NH), 1651 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{18}H_{18}ClFN_2NaO_7S$ $[M+Na]^+$: 483.0405, found: 483.0406.

(*E*)-*N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-2-naphthohydrazide (**3r**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 2-naphthhydrazide (**2r**, 6.2 mg, 33.1 µmol). Yield: 3.9 mg (25%). $[\alpha]_D^{20}$ +81.6 (*c* 0.16, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.65 (m, 1H, H-5), 3.69–3.82 (m, 3H, H-4, H-6a, H-6b), 3.93 (dd, *J* 2.1, 9.4 Hz, 1H, H-3), 4.09 (s, 1H, H-2), 5.58 (s, 1H, H-1), 7.45 (t, *J* 8.2 Hz, 1H, Ar-H), 7.53 (d, *J* 8.5 Hz, 1H, Ar-H), 7.57–7.66 (m, 2H, Ar-H), 7.80 (d, *J* 11.9 Hz, 1H, Ar-H), 7.94–8.06 (m, 4H, Ar-H), 8.32 (s, 1H, *H*C=N), 8.50 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, CD_3OD): δ 62.6 (C-6), 68.2 (C-4), 71.8 (C-2), 72.3 (C-3), 76.1 (C-5), 101.2 (C-1), 115.5 (d, $J_{C,F}$ = 20 Hz), 119.5, 125.0, 126.2, 128.1, 128.9, 129.2, 129.5, 129.6, 130.1, 131.0 (d, $J_{C,F}$ 7 Hz), 131.3, 134.0, 136.6, 147.5 (d, $J_{C,F}$ 11 Hz; 15C, Ar-C), 149.0 (H*C*=N), 154.6 (d, $J_{C,F}$ 246 Hz, Ar-C), 167.1 (C=O); IR (KBr): ν 3422 (vs, OH, NH), 1651 (s, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{24}H_{23}FN_2NaO_7$ $[M+Na]^+$: 493.1387 found: 493.1388.

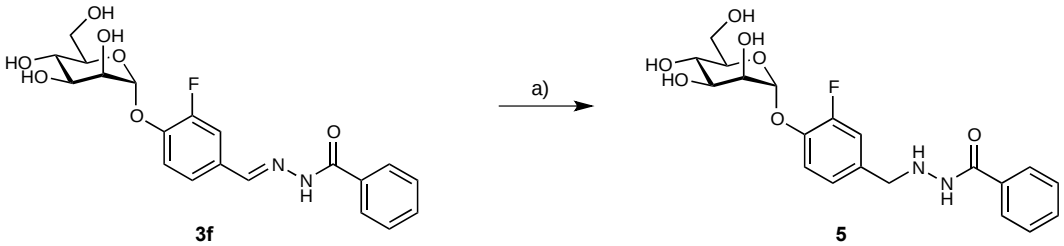
***(E)*-*N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]-4-(trifluoromethyl)benzo-hydrazide** (**3s**)**.** Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 4-(trifluoromethyl)benzhydrazide (**2s**, 6.8 mg, 33.1 µmol). Yield: 8.6 mg (53%). $[\alpha]_D^{20}$ +98.8 (*c* 0.39, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.64 (d, *J* 6.2 Hz, 1H, H-5), 3.69–3.81 (m, 3H, H-4, H-6a, H-6b), 3.93 (dd, *J* 2.2, 9.3 Hz, 1H, H-3), 4.08 (s, 1H, H-2), 5.58 (s, 1H, H-1), 7.45 (t, *J* 8.2 Hz, 1H, Ar-H), 7.51 (d, *J* 8.5 Hz, 1H, Ar-H), 7.77 (d, *J* 11.8 Hz, 1H, Ar-H), 7.84 (d, *J* 8.0 Hz, 2H, Ar-H), 8.10 (d, *J* 8.0 Hz, 2H, Ar-H), 8.28 (s, 1H, *H*C=N); ^{13}C NMR (125 MHz, CD_3OD): δ 62.6 (C-6), 68.2 (C-4), 71.7 (C-2), 72.3 (C-3), 76.1 (C-5), 101.1 (C-1), 115.5 (d, $J_{C,F}$ 20 Hz), 119.4 (2C, Ar-C), 125.2 (q, $J_{C,F}$ 267 Hz, CF_3), 126.3 (d, *J* 3 Hz), 126.7 (q, *J* 3 Hz, 2C), 129.6 (2C), 130.7 (d, $J_{C,F}$ 7 Hz), 134.6 (q, $J_{C,F}$ 33 Hz), 137.9, 147.6 (d, $J_{C,F}$ 11

Hz; 9C, Ar-C), 149.7 (H*C*=N), 154.6 (d, $J_{C,F}$ 246 Hz, Ar-C), 165.6 (C=O); IR (KBr): ν 3430 (vs, OH, NH), 1663 (vs, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{21}H_{20}F_4N_2NaO_7$ $[M+Na]^+$: 511.1104 found: 511.1107.

(*E*)-4-*N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzylidene]benzo[*b*]thiophene-2-carbohydrazide (**3t**). Prepared according to general procedure A from aldehyde **1** (10 mg, 33.1 µmol) and 3-chloro-benzo[*b*]thiophene acid hydrazide (**2t**, 7.5 mg, 33.1 µmol). Yield: 4.2 mg (25%). $[\alpha]_D^{20}$ +82.4 (*c* 0.18, MeOH); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 3.38–3.51 (m, 3H, H-6a, H-6b, H-5), 3.59 (s, 1H, H-4), 3.68 (s, 1H, H-3), 3.88 (s, 1H, H-2), 4.47 (s, 1H, OH-4), 4.71–4.96 (m, 2H, OH-3, OH-6), 5.11 (s, 1H, OH-2), 5.49 (s, 1H, H-1), 7.35–7.69 (m, 5H, Ar-H), 8.44–8.84 (m, 3H, 2 Ar-H, H*C*=N), 12.09 (s, 1H, NH); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 60.9 (C-6), 66.5 (C-4), 69.8 (C-2), 70.5 (C-3), 75.5 (C-5), 99.8 (C-1), 114.2 (d, $J_{C,F}$ 20 Hz), 118.6, 122.4, 122.6, 123.4, 124.4, 126.0, 127.4, 127.7, 132.6, 143.5, 145.4, 147.5, 152.5 (d, $J_{C,F}$ 245 Hz; 15C, Ar-C, H*C*=N), 160.2 (C=O); IR (KBr): ν 3460 (vs, OH, NH), 1656 (vs, C=N-NH-C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{22}H_{20}ClFN_2NaO_7S$ $[M+Na]^+$: 533.0561 found: 533.0562.

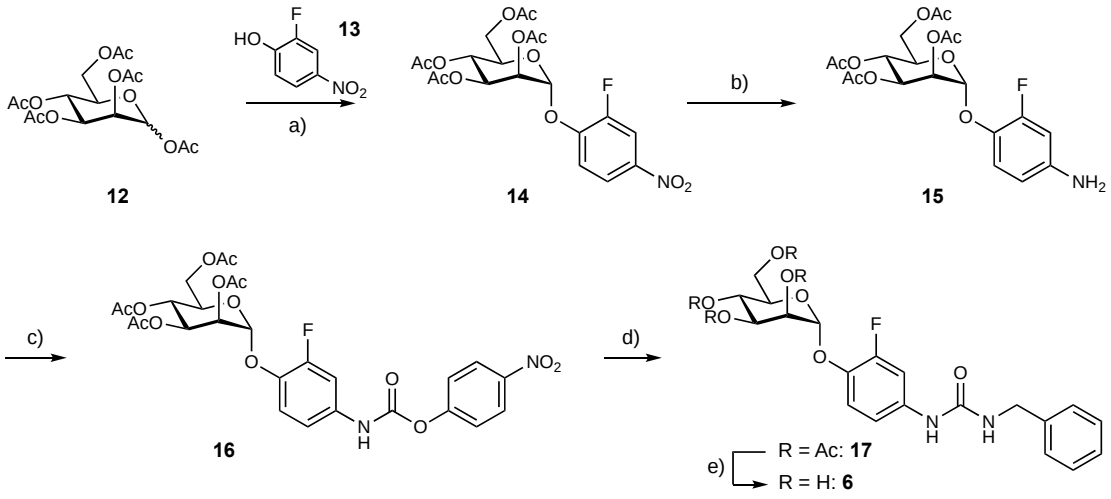
Synthesis of bioisosteres

General procedure B for deprotection of acetylated mannosides. Protected mannosides were dissolved in dry MeOH (2 mL) under argon atmosphere and freshly prepared 1 M NaOMe (100 µL) was added. The mixtures were stirred at r.t. for 30-45 min, until TLC (CH_2Cl_2/MeOH, 9:1) showed no remaining starting material. The mixtures were neutralized with amberlite ion-exchange resin (H^+-form, IR120, Sigma Aldrich), filtered, and concentrated *in vacuo*. The residues were purified by MPLC (RP-18; H_2O/MeCN, 95:5 to 20:80) to yield 60-82% of the desired products.



Scheme 2. Reagents and conditions: a) $NaBH_3CN$, aq. HCl, MeOH, r.t., 23 h, 95%.

***N*'-[3-Fluoro-4-(α-D-mannopyranosyloxy)benzyl]benzohydrazide** (**5**). Compound **3f** (4.5 mg, 10.7 µmol, 1 eq.) was dissolved in MeOH (3 mL) and $NaBH_3CN$ (6.7 mg, 107 µmol, 10 eq.) and five drops of 36% aq. HCl were added. The mixture was flushed with argon for 5 min and stirred at r.t. After 23 h the mixture was neutralized with solid NaOH and concentrated. The residue was purified by MPLC on RP-18 (H_2O/MeCN, 95:5 to 20:80) to give **5** (4.3 mg, 95%). $[\alpha]_D^{20}$ +66.3 (*c* 0.22, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.64–3.79 (m, 4H, H-4, H-5, H-6a, H-6b), 3.91 (dd, *J* 3.4, 9.3 Hz, 1H, H-3), 4.00 (s, 2H, CH_2), 4.06 (dd, *J* 1.8, 3.3 Hz, 1H, H-2), 5.46 (d, *J* 1.5 Hz, 1H, H-1), 7.13 (d, *J* 8.4 Hz, 1H, Ar-H), 7.24 (dd, *J* 1.9, 11.9 Hz, 1H, Ar-H), 7.31 (t, *J* 8.4 Hz, 1H, Ar-H), 7.42 (t, *J* 7.5 Hz, 2H, Ar-H), 7.50 (m, 1H, Ar-H), 7.69–7.75 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, CD_3OD): δ = 55.5 (CH_2), 62.6 (C-6), 68.2 (C-4), 71.9 (C-2), 72.3 (C-3), 75.8 (C-5), 101.6 (C-1), 117.9 (d, $J_{C,F}$ = 19 Hz), 120.0, 126.2 (d, $J_{C,F}$ 3 Hz), 128.2, 129.5, 132.6, 134.5, 134.8 (d, $J_{C,F}$ 17 Hz), 144.8 (d, $J_{C,F}$ = 11 Hz), 154.5 (d, $J_{C,F}$ 245 Hz; 12C, Ar-C), 169.0 (C=O); IR (KBr): ν 3438 (vs, OH), 1646 (s, C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{20}H_{24}FN_2NaO_7$ $[M+Na]^+$: 445.1387, found: 445.1386.



Scheme 3. *Reagents and conditions:* a) $BF_3{\cdot}Et_2O$, CH_2Cl_2, MS 4Å, 50 °C, 21 h, 39%; b) H_2, $Pd(OH)_2$, THF/ MeOH (2:1), r.t., 2h, 75%; c) 4-nitrophenyl chloroformate, DIPEA, CH_2Cl_2, 2 h; d) benzylamine, DIPEA, THF, 2.5 h, 29% (over two steps); e) NaOMe, MeOH, r.t., 45 min, 70%.

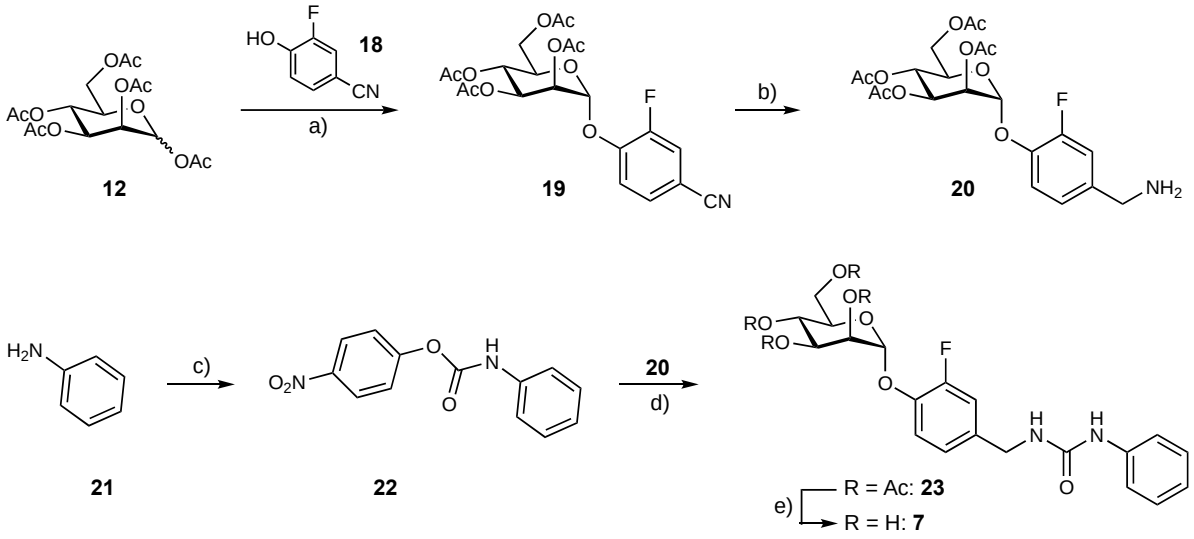
2-Fluoro-4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**14**)**.** In a two-necked flask, activated MS 4Å (ca. 500 mg), peracetylated D-mannose (**12**, 500 mg, 1.28 mmol, 1.2 eq.), 3-fluoro-4-nitrophenol (**13**, 168 mg, 1.07 mmol, 1.0 eq.), and dry CH_2Cl_2 (10 mL) were mixed and cooled down in an ice bath. Under argon atmosphere, $BF_3{\cdot}Et_2O$ (395 µL, 3.20 mmol, 3.0 eq.) was added dropwise and the reaction heated to 50 °C. The mixture was refluxed for 21 h. Then it was cooled down to r.t., diluted with EtOAc, and filtered over celite. The filtrate was subsequently washed with satd. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by MPLC on silica (petroleum ether/EtOAc, 1:0 to 4:6) to give **14** (241 mg, 39%). $[\alpha]_D^{20}$ +92.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.03, 2.05, 2.07, 2.22 (4 s, 12H, 4Ac-CH_3), 4.06–4.15 (m, 2H, H-5, H-6a), 4.27 (dd, *J* 5.9, 12.8 Hz, 1H, H-6b), 5.39 (t, *J* 9.9 Hz, 1H, H-4), 5.52 (dd, *J* 1.8, 3.5 Hz, 1H, H-2), 5.55 (dd, *J* 3.5, 9.9 Hz, 1H, H-3), 5.65 (d, *J* 1.5 Hz, 1H, H-1), 7.36 (m, 1H, Ar-H), 8.02–8.08 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.63, 20.64, 20.65, 20.8 (4 Ac-CH_3), 61.9 (C-6), 65.6 (C-4), 68.4 (C-3), 68.9 (C-2), 70.2 (C-5), 96.9 (C-1), 113.0 (d, $J_{C,F}$ 23 Hz), 117.1 (d, $J_{C,F}$ 1 Hz), 120.5 (d, $J_{C,F}$ 4 Hz), 143.0 (d, $J_{C,F}$ 7 Hz), 148.8 (d, $J_{C,F}$ 11 Hz), 151.9 (d, $J_{C,F}$ 254 Hz; 6C, Ar-C), 169.6, 169.8, 169.9, 170.4 (4 C=O); ESI-MS: *m/z:* Calcd for $C_{20}H_{22}FNNaO_{12}$ $[M+Na]^+$: 510.10, found: 510.14.

4-Amino-2-fluorophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**15**)**.** A two-necked flask equipped with a magnetic stirrer was charged with **14** (241 mg, 0.527 mmol). Under argon atmosphere, THF/MeOH (2:1, 15 mL) and $Pd(OH)_2$/C (25 mg) were added. The flask was evacuated five times and filled with H_2. Under hydrogen atmosphere, the mixture was stirred at r.t. until TLC (CH_2Cl_2/MeOH, 8:2) indicated completion of the reaction. The mixture was filtered over celite and concentrated. Purification by MPLC (petroleum ether/EtOAc, 1:0 to 0:1) gave **15** (181 mg, 75%). $[\alpha]_D^{20}$ +74.5 (*c* 2.01, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.97, 2.00, 2.01, 2.12 (4 s, 12H, 4 Ac-CH_3), 3.71 (s, 2H, NH_2), 4.07 (dd, *J* 1.8, 12.1 Hz, 1H, H-6a), 4.18–4.28 (m, 2H, H-5, H-6b), 5.22 (s, 1H, H-1), 5.29 (t, *J* 9.9 Hz, 1H, H-4), 5.44 (dd, *J* 1.7, 3.3 Hz, 1H, H-2), 5.47 (dd, *J* 3.4, 9.9 Hz, 1H, H-3), 6.29 (m, 1H, Ar-H), 6.37 (dd, *J* 2.6, 12.5 Hz, 1H, Ar-H), 6.86 (t, *J* 8.8 Hz, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.46, 20.49, 20.6 (4C, 4 Ac-CH_3), 62.1 (C-6), 65.8 (C-4), 68.7 (C-3), 69.2 (2C, C-3, C-5), 98.6 (d, $J_{C,F}$ 1 Hz, C-1), 103.3 (d, $J_{C,F}$ 22 Hz), 110.2 (d, $J_{C,F}$ 3 Hz), 121.4 (d, $J_{C,F}$ 2 Hz), 134.9 (d, $J_{C,F}$ 12 Hz), 144.1 (d, $J_{C,F}$ 10 Hz), 154.3 (d, $J_{C,F}$ 246 Hz; 6C, Ar-C), 169.6, 169.7, 169.8, 170.4 (4 C=O); ESI-MS: *m/z:* Calcd for $C_{20}H_{24}FNNaO_{10}$ $[M+Na]^+$: 480.13, found: 480.13.

4-Nitrophenyl (4'-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3'-fluorophenyl)carbamate (**16**)**.** In a two-necked flask, 4-nitrophenyl chloroformate (20.7 mg, 0.103 mmol, 1 eq.) was dissolved in dry CH_2Cl_2 (2 mL). Under argon atmosphere, a solution of **15** (47 mg, 0.103 mmol, 1 eq.) and DIPEA (17.6 µL, 0.103 mmol, 1 eq.) in dry CH_2Cl_2 (2 mL) was added dropwise. The mixture was stirred at r.t. for 2 h, until TLC (petroleum ether/EtOAc, 1:1) showed completion of the reaction. The mixture was diluted with EtOAc and washed with 1 M aq. HCl. The organic layer was dried over Na_2SO_4, filtered, and concentrated to give **16** (quant.) which was used without further purification. ESI-MS: *m/z:* Calcd for $C_{27}H_{27}FN_2NaO_{14}$ $[M+Na]^+$: 645.13, found: 645.16.

1-Benzyl-3-[4'-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3'-fluorophenyl]urea (**17**)**.** Crude **16** was dissolved in dry THF (2 mL) and benzylamine (11.3 mg, 0.103 mmol, 1 eq.) and DIPEA (17.6 µL, 0.103 mmol, 1 eq.) were added. The reaction was stirred for 1.5 h, until TLC (CH_2Cl_2/MeOH, 9:1) indicated full consumption of the starting material. The mixture was concentrated, dissolved in EtOAc, washed with 1 M aq. HCl and the aqueous layer re-extracted with EtOAc (3x). The organic layer was dried over Na_2SO_4, filtered and the solvent removed. Purification by MPLC (petroleum ether/EtOAc, 1:0 to 0:1) gave **17** (17.1 mg, 29% over two steps). $[\alpha]_D^{20}$ +35.8 (*c* 0.89, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.01, 2.02, 2.06, 2.18 (4 s, 12H, 4 Ac-CH_3), 4.08 (dd, *J* 3.4, 13.2 Hz, 1H, H-6a), 4.21–4.28 (m, 2H, H-6b, H-5), 4.37 (d, *J* 5.7 Hz, 2H, CH_2), 5.32–5.38 (m, 2H, H-4, H-1), 5.41 (t, *J* 5.6 Hz, 1H, NH), 5.49 (dd, *J* 1.8, 3.4 Hz, 1H, H-2), 5.52 (dd, *J* 3.5, 9.9 Hz, 1H, H-3), 6.91 (dd, *J* 1.2, 8.9 Hz, 1H, Ar-H), 6.94 (s, 1H, NH), 7.00 (t, *J* 8.8 Hz, 1H, Ar-H), 7.21–7.27 (m, 4H, Ar-H), 7.27–7.32 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.65, 20.66, 20.8 (4C, 4 Ac-CH_3), 44.2 (CH_2), 62.1 (C-6), 65.8 (C-3), 68.9 (C-2), 69.3 (C-4), 69.5 (C-5), 98.1 (C-1), 109.1 (d, $J_{C,F}$ 23 Hz), 115.6 (d, $J_{C,F}$ 3 Hz), 120.1 (d, $J_{C,F}$ 2 Hz), 127.4, 127.5, 128.7, 135.5 (d, $J_{C,F}$ 9 Hz), 138.6, 138.8 (d, $J_{C,F}$ 12 Hz), 153.5 (d, $J_{C,F}$ 247 Hz; 12C, Ar-C), 155.4 (NC=O), 169.8, 170.0, 170.7 (4C, 4 C=O); ESI-MS: *m/z:* Calcd for $C_{28}H_{31}FN_2Na\ O_{11}$ $[M+Na]^+$: 613.18, found: 613.22.

1-Benzyl-3-[3'-fluoro-4'-(α-D-mannopyranosyloxy)phenyl]urea (**6**)**.** Prepared according to general procedure B from **17** (17.7 mg, 30.0 µmol). Yield: 8.8 mg (70%). $[\alpha]_D^{20}$ +109.9 (*c* 0.44, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.70–3.81 (m, 4H, H-4, H-5, H-6a, H-6b), 3.90 (m, 1H, H-3), 4.06 (dd, *J* 1.8, 3.3 Hz, 1H, H-2), 4.37 (s, 2H, CH_2), 5.35 (d, *J* 1.7 Hz, 1H, H-1), 6.95 (ddd, *J* 1.3, 2.4, 8.9 Hz, 1H, Ar-H), 7.19–7.27 (m, 2H, Ar-H), 7.29–7.34 (m, 4H, Ar-H), 7.38 (dd, *J* 2.5, 13.3 Hz, 1H, Ar-H); ^{13}C NMR (125 MHz, CD_3OD): δ 44.5 (CH_2), 62.7 (C-6), 68.3 (C-4), 71.9 (C-2), 72.3 (C-3), 75.7 (C-5), 102.3 (C-1), 108.8 (d, $J_{C,F}$ 24 Hz), 115.7 (d, $J_{C,F}$ 3 Hz), 121.2 (d, $J_{C,F}$ 2 Hz), 128.1, 128.3, 129.6, 137.1 (d, $J_{C,F}$ 10 Hz), 140.4 (d, $J_{C,F}$ 11 Hz), 140.9, 154.7 (d, $J_{C,F}$ 243 Hz; 12C, Ar-C), 158.0 (C=O); IR (KBr): ν 3347 (vs, OH), 1635 (s, C=O), 1515 (vs, NH) cm^{-1}; HRMS: *m/z:* Calcd for $C_{20}H_{24}FN_2NaO_7$ $[M+Na]^+$: 445.1387, found: 445.1392.



Scheme 4. *Reagents and conditions:* a) $BF_3{\cdot}Et_2O$, CH_2Cl_2, MS 4Å, 50 °C, 29 h, 60%; b) H_2, $Pd(OH)_2$, THF/MeOH (2:1), r.t., 2 h, 64%; c) 4-nitrophenyl chloroformate, DIPEA, CH_2Cl_2, r.t., 22 h, 53%; d) DIPEA, THF, r.t., 2 h, 19%; e)NaOMe/MeOH, r.t., 45 min, 83%.

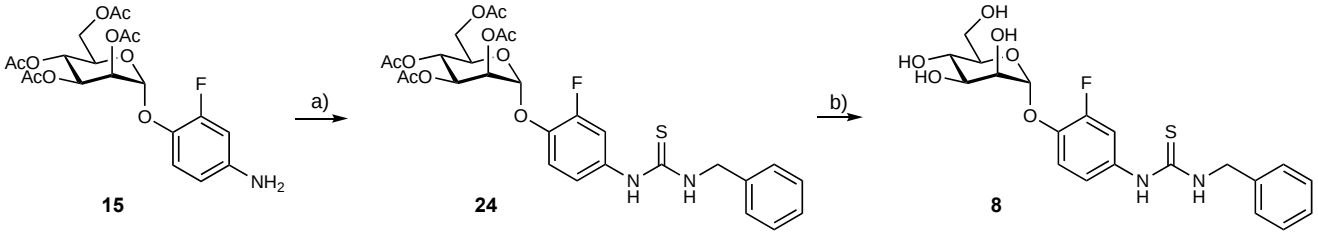
4'-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3'-fluorobenzonitrile (**19**)**.** In a two-necked flask, activated MS 4Å (500 mg), peracetylated D-mannose (**12**, 500 mg, 1.28 mmol, 1.2 eq.), 3-fluoro-4-hydroxybenzonitrile (**18**, 146 mg, 1.07 mmol, 1.0 eq.), and dry CH_2Cl_2 (10 mL) were mixed and cooled down in an ice bath. Under argon atmosphere, $BF_3{\cdot}Et_2O$ (395 µL, 3.20 mmol, 3 eq.) was added slowly and the reaction heated to 50 °C. The mixture was refluxed for 29 h. Then, it was diluted with EtOAc, filtered over celite, and subsequently washed with satd. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by MPLC (toluene/EtOAc, 1:0 to 6:4) to give **19** (356 mg, 60%). $[\alpha]_D^{20}$ +88.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.03, 2.04, 2.07, 2.21 (4 s, 12H, 4 Ac-CH_3), 4.06–4.14 (m, 2H, H-5, H-6a), 4.27 (dd, *J* 6.0, 12.9 Hz, 1H, H-6b), 5.38 (t, *J* 9.9 Hz, 1H, H-4), 5.51 (dd, *J* 1.8, 3.4 Hz, 1H, H-2), 5.53 (dd, *J* 3.5, 9.9 Hz, 1H, H-3), 5.63 (d, *J* 1.4 Hz, 1H, H-1), 7.34 (t, *J* 8.3 Hz, 1H, Ar-H), 7.42–7.48 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.37, 20.39, 20.41, 20.5 (4 Ac-CH_3), 61.7 (C-6), 65.4 (C-4), 68.2 (C-3), 68.7 (C-2), 69.9 (C-5), 96.6 (C-1), 107.0 (d, $J_{C,F}$ 8 Hz), 117.1 (d, $J_{C,F}$ 2 Hz), 120.3 (d, $J_{C,F}$ 22 Hz), 129.2 (d, $J_{C,F}$ 4 Hz), 147.2 (d, $J_{C,F}$ 11 Hz), 152.2 (d, $J_{C,F}$ 252 Hz; 7C, Ar-C, CN), 169.4, 169.5, 169.6, 170.1 (4 C=O); IR (KBr): ν 2232 (w, CN), 1751 (vs, C=O) cm^{-1}; ESI-MS: *m/z:* Calcd for $C_{21}H_{22}FNNaO_{10}$ $[M+Na]^+$: 490.11, found: 490.06.

4'-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3'-fluorophenylmethanamine (**20**)**.** A two-neckeds flask was charged with **19** (50 mg, 0.107 mmol) and a magnetic stirrer. Under argon atmosphere, THF/MeOH (6 mL, 2:1) and $Pd(OH)_2$/C (15 mg) were added. The flask was evacuated five times and filled with H_2. Under hydrogen atmosphere, the mixture was stirred at r.t. until TLC (CH_2Cl_2/MeOH, 8:2) indicated completion of the reaction. The mixture was filtered over celite, and concentrated. Purification by MPLC (CH_2Cl_2/MeOH, 1:0 to 8:2) gave **20** (32.2 mg, 64%). $[\alpha]_D^{20}$ +93.4 (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.03, 2.04, 2.07, 2.20 (4 s, 12H, 4 Ac-CH_3), 3.83 (s, 2H, CH_2), 4.10 (d, *J* 10.3 Hz, 1H, H-6a), 4.22–4.30 (m, 2H, H-5, H-6b), 5.37 (t, *J* 10.0 Hz, 1H, H-4), 5.46 (d, *J* 1.6 Hz, 1H, H-1), 5.52 (dd, *J* 1.8, 3.4 Hz, 1H, H-2), 5.57 (dd, *J* 3.5, 10.0 Hz, 1H, H-3), 7.02 (d, *J* 8.3 Hz, 1H, Ar-H), 7.09–7.16 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.60, 20.63, 20.8 (4C, 4 Ac-CH_3), 45.3 (CH_2), 62.1 (C-6), 65.8 (C-4), 68.7 (C-3), 69.3 (C-2), 69.5 (C-5), 97.7 (C-1), 115.5 (d, $J_{C,F}$ 19 Hz), 119.4 (d, $J_{C,F}$ 1 Hz), 122.9 (d, $J_{C,F}$ 3 Hz), 139.7 (d, $J_{C,F}$ 6 Hz), 141.9 (d, $J_{C,F}$ 11 Hz), 153.4 (d, $J_{C,F}$ 248 Hz; 6C, Ar-C), 160.7, 169.8, 169.8, 170.5 (4 C=O); ESI-MS: *m/z:* Calcd for $C_{21}H_{27}FNO_{10}$ $[M+H]^+$: 472.16, found: 472.13.

4-Nitrophenyl phenylcarbamate (**22**)**.** To a mixture of aniline (**21**, 49.0 µL, 0.537 mmol) and 4-nitrophenyl chloroformate (108 mg, 0.537 mmol) in THF (2 mL) was added DIPEA (91.9 µL, 0.537 mmol). The mixture was stirred at r.t. After 22 h, TLC (toluene/EtOAc, 1:1) indicated no remaining starting materials. The mixture was diluted with EtOAc and washed with 1 M aq. HCl. The aqueous layer was extracted with EtOAc, the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by MPLC (toluene/EtOAc, 1:0 to 1:1) to give **22** (72.8 mg, 52%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.06 (s, 1H, NH), 7.16 (t, *J* 7.4 Hz, 1H, Ar-H), 7.34–7.41 (m, 4H, Ar-H), 7.45 (d, *J* 7.8 Hz, 2H, Ar-H), 8.25–8.31 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 119.0 (2C), 122.1 (2C), 124.6, 125.2 (2C), 129.3 (2C), 136.6, 145.1 (12C, Ar-C), 150.1 (C=O), 155.4 (Ar-C).

1-[4'-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3'-fluoro]-3-phenylurea (**23**). A mixture of **20** (50 mg, 107 µmol), DIPEA (18.2 µL, 107 µmol) and **22** (27.4 mg, 107 µmol) in dry THF (2 mL) was stirred at r.t. for 2 h, until TLC (CH_2Cl_2/MeOH, 9:1) showed no remaining starting material. The mixture was diluted with EtOAc and washed with 1 M aq. HCl. The aqueous layer was extracted three times with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by MPLC (CH_2Cl_2/MeOH, 1:0 to 9:1) to give **23** (12.1 mg, 19%). $[\alpha]_D^{20}$ +51.2 (*c* 0.61, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.02, 2.03, 2.06, 2.19 (4 s, 12H, 4 Ac-CH_3), 4.08 (dd, *J* 2.0, 12.1 Hz, 1H, H-6a), 4.20 (ddd, *J* 2.0, 5.2, 10.0 Hz, 1H, H-5), 4.25 (dd, *J* 5.2, 12.1 Hz, 1H, H-6b), 4.32 (d, *J* 5.9 Hz, 2H, CH_2), 5.33–5.39 (m, 2H, H-4, NH), 5.42 (d, *J* 1.5 Hz, 1H, H-1), 5.49 (dd, *J* 1.8, 3.4 Hz, 1H, H-2), 5.54 (dd, *J* 3.5, 10.0 Hz, 1H, H-3), 6.80 (s, 1H, NH), 6.96 (d, *J* 8.4 Hz, 1H, Ar-H), 7.03 (dd, *J* 1.8, 11.4 Hz, 1H, Ar-H), 7.05–7.11 (m, 2H, Ar-H), 7.24–7.30 (m, 4H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 20.7, 20.8 (4C, 4 Ac-CH_3), 43.2 (CH_2), 62.1 (C-6), 65.8 (C-4), 68.8 (C-3), 69.2 (C-2), 69.6 (C-5), 97.6 (C-1), 115.8 (d, $J_{C,F}$ 19 Hz), 119.4, 121.1, 123.3 (d, $J_{C,F}$ 4 Hz), 124.1, 129.3, 135.9 (d, $J_{C,F}$ 6 Hz), 138.2, 142.3 (d, $J_{C,F}$ 11 Hz), 153.4 (d, $J_{C,F}$ 249 Hz; 12C, Ar-C), 155.8 (C=O(NH)$_2$), 169.7, 169.9, 170.0, 170.6 (4 C=O); ESI-MS: *m/z:* Calcd for $C_{28}H_{31}FN_2NaO_{11}$ $[M+Na]^+$: 613.18, found: 613.31.

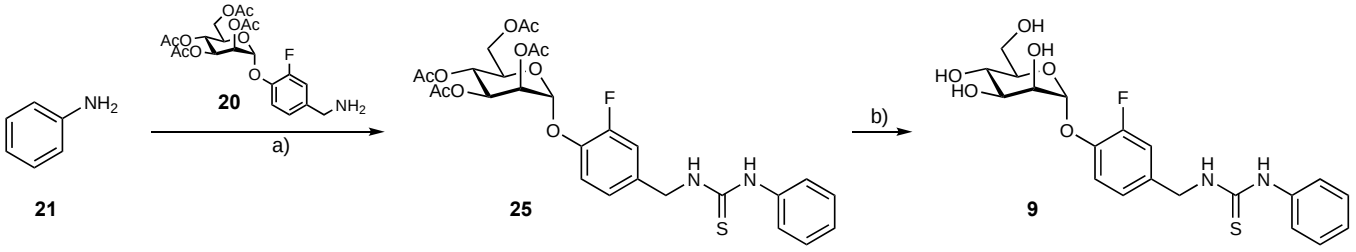
1-(3'-Fluoro-4'-α-D-mannopyranosyloxy)-3-phenylurea (**7**)**.** Prepared according to general procedure B from **23** (12.1 mg, 20.5 µmol). Yield: 7.2 mg (83%). $[\alpha]_D^{20}$ +88.1 (*c* 0.36, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.66–3.80 (m, 4H, H-4, H-5, H6a, H-6b), 3.91 (dd, *J* 3.4, 9.2 Hz, 1H, H-3), 4.06 (dd, *J* 1.8, 3.3 Hz, 1H, H-2), 5.44 (d, *J* 1.6 Hz, 1H, H-1), 6.97 (t, *J* 7.4 Hz, 1H, Ar-H), 7.07 (d, *J* 8.4 Hz, 1H, Ar-H), 7.11 (dd, *J* 1.9, 11.9 Hz, 1H, Ar-H), 7.21–7.28 (m, 2H, Ar-H), 7.31 (t, *J* 8.4 Hz, 1H, Ar-H), 7.35 (dd, *J* 1.0, 8.5 Hz, 2H, Ar-H); ^{13}C NMR (125 MHz, CD_3OD): δ 43.6 (CH_2), 62.6 (C-6), 68.2 (C-4), 71.9 (C-2), 72.3 (C-3), 75.8 (C-5), 101.7 (C-1), 116.2 (d, $J_{C,F}$ 19 Hz), 120.3, 123.6, 124.3 (d, $J_{C,F}$ 3 Hz), 129.8, 137.0 (d, $J_{C,F}$ 6 Hz), 140.8, 144.4 (d, $J_{C,F}$ 11 Hz), 154.6 (d, $J_{C,F}$ 246 Hz; 12 C, Ar-C), 158.2 (C=O); IR (KBr): ν 3371 (vs, OH), 1651 (s, C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{20}H_{24}FN_2NaO_7$ $[M+Na]^+$: 445.1387, found: 445.1385.



Scheme 5. *Reagents and conditions:* a) i. TCDI, CH_2Cl_2, r.t., 17 h; ii. benzylamine, CH_2Cl_2, r.t., 3 h, 52% (over two steps); b) NaOMe, MeOH, 0 °C, 45 min, 71%.

1-Benzyl-3-[4'-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3'-fluorophenyl]thiourea (**24**). Compound **15** (47.0 mg, 0.103 mmol) and 1,1'-thiocarbonyldiimidazole (TCDI; 18.3 mg, 0.103 mmol.) were dissolved in dry CH_2Cl_2 (2 mL). The mixture was stirred at r.t. for 17 h. Then benzylamine (11.2 µL, 0.103 mmol) was added and the mixture was stirred for 3 h until TLC (petroleum ether/EtOAc, 1:1) showed no remaining starting material. The mixture was diluted with CH_2Cl_2, and washed with 1 M aq. HCl, satd. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by MPLC (petroleum ether/EtOAc, 1:0 to 0:1) to give **24** (32.3 mg, 52%). $[\alpha]_D^{20}$ +51.6 (*c* 1.07, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.96, 1.99, 2.02, 2.15 (4 s, 12H, 4 Ac-CH_3), 4.04 (dd, *J* 2.4, 12.1 Hz, 1H, H-6a), 4.12 (ddd, *J* 2.3, 5.2, 10.1 Hz, 1H, H-5), 4.20 (dd, *J* 5.2, 12.2 Hz, 1H, H-6b), 4.81 (d, *J* 5.2 Hz, 2H, CH_2), 5.32 (t, *J* 10.1 Hz, 1H, H-4), 5.41 (s, 1H, H-1), 5.43 (dd, *J* 1.8, 3.3 Hz, 1H, H-2), 5.48 (dd, *J* 3.4, 10.0 Hz, 1H, H-3), 6.23 (s, 1H, NH), 6.92 (d, *J* 8.7 Hz, 1H, Ar-H), 7.02 (dd, *J* 2.2, 11.0 Hz, 1H, Ar-H), 7.16 (t, *J* 8.7 Hz, 1H, Ar-H), 7.22–7.27 (m, 3H, Ar-H), 7.27–7.32 (m, 2H, Ar-H), 7.96 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.59, 20.61, 20.62, 20.8 (4 Ac-CH_3), 49.4 (CH_2), 62.1 (C-6), 65.7 (C-4), 68.5 (C-3), 69.1 (C-2), 69.7 (C-5), 97.5 (C-1), 114.4 (d, $J_{C,F}$ 20 Hz), 119.9 (d, $J_{C,F}$ 2 Hz), 121.6 (d, $J_{C,F}$ 4 Hz), 127.7, 127.9, 128.8, 137.0, 142.4 (d, $J_{C,F}$ 11 Hz), 153.4 (d, $J_{C,F}$ 252 Hz; 12C, Ar-C), 169.7, 169.8, 169.9, 170.4 (4 C=O), 181.1 (C=S); ESI-MS: *m/z:* Calcd for $C_{28}H_{32}FN_2O_{10}S$ $[M+H]^+$: 607.18, found: 607.30.

1-Benzyl-3-[3-fluoro-4-(α-D-mannopyranosyloxy)phenyl]thiourea (**8**)**.** Prepared according to general procedure B from **24** (32.2 mg, 53.1 µmol). Yield: 16.7 mg (71%). $[\alpha]_D^{20}$ +95.6 (*c* 0.84, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.65–3.81 (m, 4H, H-4, H-5, H-6a, H-6b), 3.90 (dd, *J* 3.4, 9.1 Hz, 1H, H-3), 4.06 (dd, *J* 1.8, 3.3 Hz, 1H, H-2), 4.80 (s, 2H, CH_2), 5.45 (d, *J* 1.6 Hz, 1H, H-1), 7.03 (ddd, *J* 1.4, 2.3, 8.8 Hz, 1H, Ar-H), 7.25 (m, 1H, Ar-H), 7.27–7.36 (m, 6H, Ar-H); ^{13}C NMR (125 MHz, CD_3OD): δ 49.0 (CH_2), 62.7 (C-6), 68.2 (C-4), 71.8 (C-2), 72.3 (C-3), 75.9 (C-5), 101.7 (C-1), 114.5 (d, $J_{C,F}$ 21 Hz), 120.4, 121.9 (d, $J_{C,F}$ 5 Hz), 128.2, 128.6, 129.5, 135.1 (d, $J_{C,F}$ 9 Hz), 139.9, 143.2 (d, $J_{C,F}$ 11.0 Hz), 154.3 (d, $J_{C,F}$ 246 Hz; 12C, Ar-C), 182.9 (C=S); IR (KBr): ν 3295 (vs, OH), 1563 (vs), 1509 (vs) cm^{-1}; HRMS: *m/z:* Calcd for $C_{20}H_{24}FN_2NaO_6S$ $[M+Na]^+$: 461.1159 found: 461.1161.

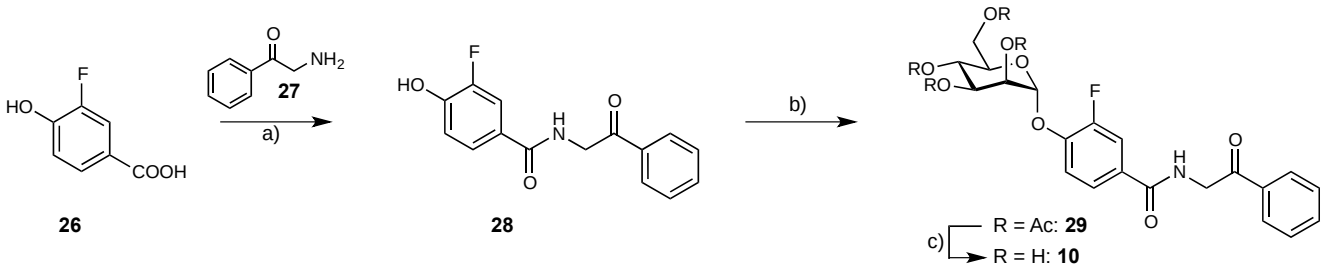


Scheme 6. *Reagents and conditions:* a) i. TCDI, CH_2Cl_2, r.t., 18 h; ii. **20**, CH_2Cl_2, r.t., 6 h, 7% (over two steps); b) NaOMe, MeOH, r.t., 45 min, 66%.

1-[4'-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3'-fluorobenzyl]-3-phenylthiourea (**25**)**.** 1,1'-Thiocarbonyldiimidazole (18.9 mg, 0.106 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and **21** (9.7 µL, 0.106 mmol) was added. The mixture was stirred at r.t. for 18 h until TLC (toluene/EtOAc, 1:1) showed no remaining aniline. Then, **20** (50 mg, 0.106 mmol) in dry CH_2Cl_2 (2 mL) was added and the mixture stirred for another 6 h. Then, it was diluted with CH_2Cl_2 and subsequently washed with 1 M aq. HCl, satd. aq. $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, filtered, and the solvent removed. The residue was purified by MPLC (toluene/EtOAc, 1:0 to 1:1) to give **25** (4.8 mg, 7 %). $[\alpha]_D^{20}$ +49.8 (*c* 0.24, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.03, 2.06, 2.19 (3 s, 12H, 4 Ac-CH_3), 4.09 (dd, *J* 1.9, 12.2 Hz, 1H, H-6a), 4.20 (ddd, *J* 1.8, 5.2, 9.9 Hz, 1H, H-5), 4.26 (dd, *J* 5.3, 12.1 Hz, 1H, H-6b), 4.83 (d, *J* 5.7 Hz, 2H, CH_2), 5.36 (t, *J* 10.0 Hz, 1H, H-4), 5.45 (s, 1H, H-1), 5.49 (m, 1H, H-2), 5.55 (dd, *J* 3.5, 10.0 Hz, 1H, H-3), 6.22 (s, 1H, NH), 7.01 (d, *J* 8.4 Hz, 1H, Ar-H), 7.09 (dd, *J* 1.5, 11.3

Hz, 1H, Ar-H), 7.12 (t, *J* 8.2 Hz, 1H, Ar-H), 7.15–7.26 (m, 2H, Ar-H), 7.34 (m, 1H, Ar-H), 7.44 (t, *J* 7.8 Hz, 2H, Ar-H), 7.72 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.66, 20.67, 20.68, 20.8 (4 Ac-CH_3), 48.3 (CH_2), 62.1 (C-6), 65.8 (C-4), 68.7 (C-3), 69.3 (C-2), 69.6 (C-5), 97.6 (C-1), 116.1 (d, $J_{C,F}$ 19 Hz), 119.3, 123.6 (d, $J_{C,F}$ 4 Hz), 125.6, 127.8, 128.8, 130.4, 134.0 (d, *J* 6 Hz), 142.8 (d, $J_{C,F}$ 11 Hz), 153.4 (d, $J_{C,F}$ 249 Hz; 12C, Ar-C), 169.7, 169.8, 169.9, 170.5 (4 C=O), 181.3 (C=S); ESI-MS: *m/z:* Calcd for $C_{28}H_{31}FN_2NaO_{10}S$ $[M+H]^+$: 629.16, found: 629.15.

1-(3-Fluoro-4-α-D-mannopyranosyloxybenzyl)-3-phenylthiourea (**9**). Prepared according to general procedure B from **25** (4.6 mg, 7.9 µmol). Yield: 2.3 mg (66%). $[\alpha]_D^{20}$ +61.4 (*c* 0.12, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 3.66–3.79 (m, 4H, H-4, H-5, H-6a, H-6b), 3.91 (dd, *J* 3.4, 9.1 Hz, 1H, H-3), 4.06 (dd, *J* 1.8, 3.4 Hz, 1H, H-2), 4.76 (s, 2H, CH_2), 5.45 (d, *J* 1.7 Hz, 1H, H-1), 7.09 (d, *J* 8.4 Hz, 1H, Ar-H), 7.15 (dd, *J* 2.0, 11.9 Hz, 1H, Ar-H), 7.21 (m, 1H, Ar-H), 7.28–7.39 (m, 5H, Ar-H); ^{13}C NMR (125 MHz, CD_3OD): δ 48.2 (CH_2), 62.6 (C-6), 68.2 (C-4), 71.9 (C-2), 72.3 (C-3), 75.8 (C-5), 101.7 (C-1), 116.6 (d, $J_{C,F}$ 19 Hz), 120.2 (d, $J_{C,F}$ 1 Hz), 124.7 (d, $J_{C,F}$ 3 Hz), 125.9 (d, $J_{C,F}$ 3 Hz), 127.0, 130.3, 139.5, 144.4, 154.5 (d, $J_{C,F}$ 246 Hz; 12C, Ar-C), 182.8 (C=S); IR (KBr): ν 3422 (vs, OH), 1514 (m, NH) cm^{-1}; HRMS: *m/z:* Calcd for $C_{20}H_{24}FN_2NaO_6S$ $[M+Na]^+$: 461.1159 found: 461.1160.



Scheme 7. *Reagents and conditions:* a) HBTU, HOBt, DIPEA, DMF, r.t., 2.5 h, 18%; b) **11**, $BF_3{\cdot}Et_2O$, CH_2Cl_2/ MeCN, MS 4Å, 50-75 °C, 48 h, 12%; c) NaOMe, MeOH, r.t., 45 min, 60%.

3-Fluoro-4-hydroxy-*N*-(2-oxo-2-phenylethyl)benzamide (**28**). 3-Fluoro-4-hydroxybenzoic acid (**26**, 50.0 mg, 0.641 mmol, 1 eq.), HBTU (243 mg, 1.28 mmol, 2 eq.), HOBt hydrate (12% water; 98.4 mg, 0.205 mmol, 2 eq.), and 2-aminoacetophenone hydrochloride (**27**, 35.2 mg, 1.28 mmol, 2 eq.) were dissolved in anhydrous DMF (1.5 mL). Then, DIPEA (110 µL, 2.56 mmol, 4 eq.) was added and the mixture was stirred at r.t. for 2.5 h. Then, it was diluted with EtOAc and subsequently washed with 1 M aq. HCl and brine. The organic layer was dried over Na_2SO_4, filtered and the solvents were removed *in vacuo*. The residue was purified by MPLC (toluene/EtOAc, 1:0 to 1:1) to give **28** (31.8 mg, 18%). ^{1}H NMR (500 MHz, CD_3OD): δ 4.86 (s, 2H, CH_2), 6.99 (t, *J* 8.5 Hz, 1H, Ar-H), 7.53 (t, *J* 7.7 Hz, 2H, Ar-H), 7.60 (dd, *J* 2.1, 8.4 Hz, 1H, Ar-H), 7.62–7.67 (m, 2H, Ar-H), 8.02–8.08 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, CD_3OD): δ 47.7 (CH_2), 116.5 (d, $J_{C,F}$ 20 Hz), 118.5 (d, $J_{C,F}$ 3 Hz), 125.3 (d, $J_{C,F}$ 3 Hz), 126.7 (d, $J_{C,F}$ 6 Hz), 129.0, 129.9, 134.8, 136.5, 150.0 (d, $J_{C,F}$ 13 Hz), 152.4 (d, $J_{C,F}$ 242 Hz; 12C, Ar-C), 169.1 (CONH), 196.4 (C=O); ESI-MS: *m/z:* Calcd for $C_{15}H_{12}FNNaO_3$ $[M+Na]^+$: 296.07, found: 295.49.

4-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)-3-fluoro-*N*-(2-oxo-2-phenylethyl)benzamide (**29**)**.** A two-necked flask was charged with activated MS 4Å (50 mg), peracetylated D-mannose (**12**, 54.5 mg, 0.140 mmol, 1.2 eq.) and dry CH_2Cl_2 (2 mL). Under argon atmosphere, **28** (31.8 mg, 0.116 mmol, 1 eq.) in dry MeCN (2 mL) was added. The mixture was refluxed at 50 °C for 24 h, and another 24 h at 75°C. When TLC (petroleum ether/EtOAc, 1:1) showed no remaining mannose precursor, the mixture was diluted with EtOAc, filtered over celite, and washed with satd. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by MPLC (petroleum ether/EtOAc, 1:0 to 1:1) to yield **29** (8.3 mg, 12%). Unreacted **28** (15.5 mg, 49%) could be recovered. $[\alpha]_D^{20}$ +60.1 (*c* 0.42, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ

2.04, 2.04, 2.07, 2.21 (4 s, 12H, 4 Ac-CH_3), 4.09 (dd, *J* 2.1, 12.2 Hz, 1H, H-6a), 4.16 (ddd, *J* 1.9, 5.3, 9.9 Hz, 1H, H-5), 4.28 (dd, *J* 5.4, 12.3 Hz, 1H, H-6b), 4.94 (d, *J* 4.1 Hz, 2H, CH_2), 5.38 (t, *J* 10.1 Hz, 1H, H-4), 5.53 (dd, *J* 1.8, 3.3 Hz, 1H, H-2), 5.55–5.60 (m, 2H, H-3, H-1), 7.22 (s, 1H, NH), 7.28 (t, *J* 8.2 Hz, 1H, Ar-H), 7.53 (t, *J* 7.7 Hz, 2H, Ar-H), 7.62 (d, *J* 8.6 Hz, 1H, Ar-H), 7.65 (t, *J* 7.4 Hz, 1H, Ar-H), 7.70 (dd, *J* 1.9, 11.1 Hz, 1H, Ar-H), 8.03 (d, *J* 7.5 Hz, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.64, 20.65, 20.66, 20.8 (4 Ac-CH_3), 46.9 (CH_2), 62.0 (C-6), 65.7 (C-4), 68.6 (C-3), 69.1 (C-2), 69.8 (C-5), 97.0 (C-1), 116.2 (d, $J_{C,F}$ 20 Hz), 118.1, 123.4 (d, $J_{C,F}$ 4 Hz), 128.0, 129.0, 129.9 (d, $J_{C,F}$ 6 Hz), 134.2, 134.4, 146.1 (d, $J_{C,F}$ 11 Hz), 152.8 (d, $J_{C,F}$ 250 Hz; 12C, Ar-C), 165.4, 169.7, 169.8, 169.9, 170.5, 194.0 (6 C=O); ESI-MS: *m/z:* Calcd for $C_{29}H_{30}FNNaO_{12}$ $[M+Na]^+$: 626.17, found: 626.24.

3-Fluoro-4-α-D-mannopyranosyloxy-*N*-(2-oxo-2-phenylethyl)benzamide (**10**)**.** Prepared according to general procedure B from **29**. Yield: 3.6 mg (60%). $[\alpha]_D^{20}$ +88.3 (*c* 0.18, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.62 (ddd, *J* 2.4, 5.6, 9.8 Hz, 1H, H-5), 3.68–3.81 (m, 3H, H-4, H-6a, H-6b), 3.93 (dd, *J* 3.4, 9.5 Hz, 1H, H-3), 4.09 (dd, *J* 1.8, 3.4 Hz, 1H, H-2), 4.87 (s, 2H, CH_2), 5.62 (d, *J* 1.5 Hz, 1H, H-1), 7.50 (t, *J* 8.5 Hz, 1H, Ar-H), 7.55 (t, *J* 7.7 Hz, 2H, Ar-H), 7.66 (t, *J* 7.4 Hz, 1H, Ar-H), 7.68–7.74 (m, 2H, Ar-H), 8.04–8.08 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, CD_3OD): δ 62.6 (C-6), 68.2 (C-4), 71.7 (C-2), 72.3 (C-3), 76.1 (C-5), 101.0 (C-1), 116.6 (d, $J_{C,F}$ 20 Hz), 119.0, 125.2 (d, $J_{C,F}$ 4 Hz), 129.1, 130.0, 134.9, 136.5, 148.5 (d, $J_{C,F}$ 11 Hz), 153.9 (d, $J_{C,F}$ 246 Hz; 12C, Ar-C), 168.7 (CONH), 196.3 (C=O); IR (KBr): ν 3412 (vs, OH, NH), 1646 (s, C=O) cm^{-1}; HRMS: *m/z:* Calcd for $C_{21}H_{23}FNNaO_8$ $[M+Na]^+$: 458.1227, found: 458.1227.

Supplementary Material

For Surface Plasmon Resonance Experiments and Fluorescence Polarization Assay please refer to the Supporting Information for a detailed description.

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