Carbohydrates

Cellular Fucosylation Inhibitors Based on Fluorinated Fucose-1-phosphates**

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Abstract: Fucosylation of glycans impacts a myriad of physiological and pathological processes. Inhibition of fucose expression emerges as a potential therapeutic avenue for example in cancer, inflammation, and infection. In this study, we found that protected 2-fluorofucose 1-phosphate efficiently inhibits cellular fucosylation with a four to seven times higher potency than known inhibitor 2FF, independently of the anomeric stereochemistry. Nucleotide sugar analysis revealed that both the α- and β-GDP-2FF anomers are formed inside the cell. In conclusion, we developed A2FF1P and B2FF1P as potent new tools for studying the role of fucosylation in health and disease and they are potential therapeutic candidates.

L-Fucose (6-deoxy-L-galactose) is an important monosaccharide constituent of glycans and is expressed in a wide variety of organisms.[1,2] In mammals, fucosylated glycans are involved in a myriad of physiological activities such as selectin binding of leukocytes thereby mediating recruitment to sites of inflammation, tissue development via growth factor receptor and Notch signaling, or fertilization and cognitive processes.[3] Additionally, fucose sugars are involved in regulating the immune system through immune cell development and modulating Fc receptor binding to the glycosylated Fc region of IgG1 antibodies, thereby regulating antibody-dependent cellular cytotoxicity (ADCC).[1,3,4] Next to these physiological roles, aberrant fucosylation is associated with diverse pathological processes.[5,6] In particular in cancer, fucosylation can mediate pro- and anti-tumorigenic effects through modulation of growth factor receptor-mediated signaling, proliferation, endothelial to mesenchymal transition (EMT), angiogenesis, tissue invasion, metastasis, and chemotherapy resistance mechanisms.[5,6] Tools to modulate fucosylation in vitro and in vivo are therefore highly valuable for dissecting its role in biology and diseases such as cancer with potential therapeutic applications.

One of the most utilized fucosylation inhibitors is 1,3,4-tri-*O*-acetyl-2-deoxy-2-fluoro-fucose (2FF).[7] 2FF is passively transported over the cell membrane, deprotected by esterases and metabolized to its respective GDP-analogue. The active GDP-analogue decreases fucosylation by feedback inhibition of de novo biosynthesis and competitive inhibition of fucosyltransferases (Scheme 1).[7] Proof of concept studies showed that inhibiting fucosylation in vitro with 2FF, or its deacetylated form, in liver and breast cancer cells resulted in reduced proliferation and migration, and downstream growth factor activation.[8,9] In vivo studies showed that pre-treatment of HepG2 liver cancer cells reduced outgrowth after subcutaneous transplantation in mice.[8]

Oral administration of 2FF delayed tumor growth of LS174T colorectal carcinoma, breast cancer and A20 lymphoma in mice, likely due to enhanced ADCC after fucose inhibition.[10,11,12] Remarkably, oral 2FF treatment combined with immunotherapy completely protected against tumor growth due to enhanced ADCC with A20 lymphoma cells, with no apparent toxicity in mice.[10] Although these data clearly indicate the promise of 2FF as a potential therapeutic option for cancer, we found that the sensitivity of different cell lines for 2FF varied from low micromolar to high micromolar concentrations. These findings prompted us to improve the inhibitory potency of 2FF.

We recently showed that the potency of metabolic inhibitors of the sialic acid biosynthesis could be improved by chemical

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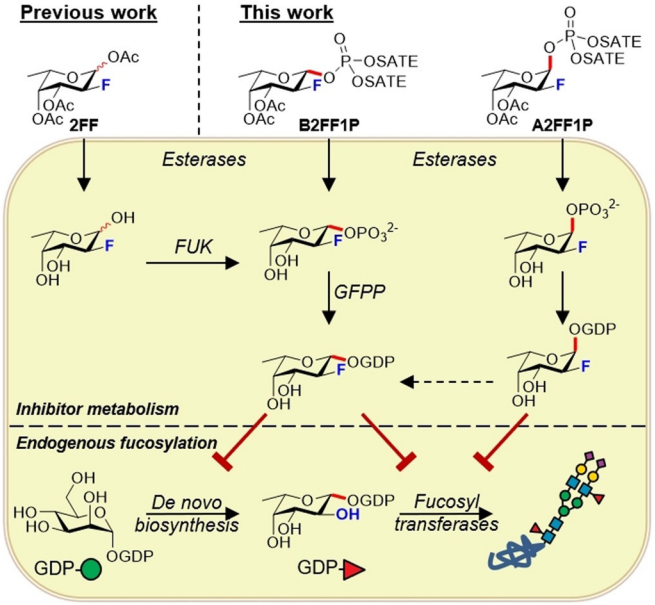
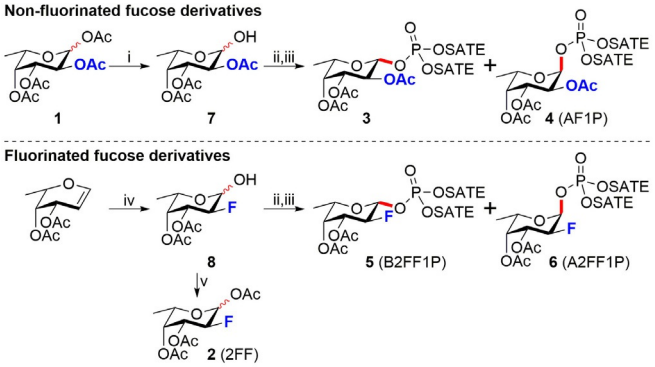


Scheme 1. Working model of fucosylation inhibitors 2FF, A2FF1P and B2FF1P. The (thio)ester protected derivatives are passively transported over the cell membrane, deprotected by esterases and metabolized to their respective GDP-analogues. The active GDP-analogues decrease fucosylation by feedback inhibition of de novo biosynthesis and competitive inhibition of fucosyltransferases. Notably, A2FF1P with an unnatural anomeric α-configuration is also converted to its GDP-analogue and inhibits fucosylation with similar potency as B2FF1P.



Scheme 2. Synthesis of **1**–**8**. i) H_2NNH_2·HOAc, DMF ii) bis(*S*-acetyl-2-thioethyl)*N*,*N*-diethylphosphoramidite, 1*H*-tetrazole, ACN; iii) mCPBA, ACN, iv) Selectfluor, DMF, water, v) Ac_2O, pyridine.

modifications enhancing their metabolic conversion into the active inhibitor inside cells.[13] We therefore hypothesized that the potency of 2FF could be improved by feeding its 1-phosphate derivative as the metabolic precursor, thereby entering the metabolic fucose pathway in a later stage (Scheme 1). Additionally, after (thio)ester deprotection the 1-phosphate derivatives are expected to be better retained inside the cell than unphosphorylated counterparts.[14] Combined, we hypothesized that these effects would lead to a more efficient conversion to GDP-Fuc2F and hence a more potent inhibition of fucosylation. Herein, we report the synthesis and evaluation of both the unnatural α-anomer and the endogenous β-anomer 1-phosphate analogues of 2FF, designated A2FF1P (**5**) and B2FF1P (**6**), respectively.

To investigate the influence of C-2 fluorination and the anomeric stereochemistry of a 1-phosphate group we prepared fucosides **1**–**6** (Scheme 2). Selective anomeric deacetylation of acetylated fucose **1** afforded lactol **7**. Anomeric phosphorylation was achieved using a phosphoramidite reagent bearing two S-acetyl-2-thioethyl (SATE) groups affording a mixture of the β- and α-phosphite. Excess phosphoramidite reagent was removed by silica gel column chromatography. Subsequently, the mixture of β- and α-phosphite triesters was oxidized to the corresponding phosphate triesters **3** and **4**. After silica column chromatography and HPLC purification only the α-anomer (**4**) was obtained in its pure form. The corresponding 2-fluoro analogues were synthesized via fluorination of acetylated fucal using Selectfluor in a mixture of water and DMF to afford lactol **8**. Acetylation of **8** afforded reference compound 2FF (**2**).[15,7] Using the aforementioned phosphorylation sequence, **8** was converted in a by silica gel column chromatography separable mixture of β-1-phosphate triester **5** (B2FF1P) and its corresponding α-anomer **6** (A2FF1P).

Next, the inhibitory potency of A2FF1P, B2FF1P and 2FF was evaluated in human THP-1, HeLa and H1299 cell lines. To this end, cells were incubated with varying concentrations of the respective compounds for 3 days. The cellular fucose expression was evaluated using flow cytometry after staining cell surface fucose residues with either the AAL or AOL lectin which preferably bind *N*-glycan terminal and core fucosides, respectively (Figure 1a; Figure S1 in Supporting Information). The EC_{50} values were determined, defined as the concentration where a 50% decrease in lectin binding compared to control was observed (Table 1). The known reference compound 2FF (**2**) was compared to its β- and α-phosphate derivative, B2FF1P (**5**) and A2FF1P (**6**). We found that B2FF1P (**5**) inhibited fucosylation in all three cell lines with 4–7 times enhanced potency compared to 2FF (**2**) (Table 1). This is in line with our hypothesis that a reduction in metabolic steps affords a more potent fucosylation inhibitor. Unexpectedly, A2FF1P (**5**) bearing the unnatural anomeric configuration also showed inhibition with a similar potency as B2FF1P (**6**). The non-fluorinated α-phosphate counterpart AF1P (**4**), did not show inhibition in all three cell lines, indicating that the orientation of the phosphate by itself is not responsible for the inhibitory effect of A2FF1P (Figure S1).

Next, a toxicity profile of the three inhibitors was established by monitoring the cell death and metabolic activity after three days of treatment (Figure 1b). 2FF, B2FF1P and A2FF1P showed no cytotoxicity up to 512 μM, however metabolic activity of cells was affected at concentrations above 128 μM for A2FF1P, but not for 2FF and B2FF1P. It has been suggested that fucosylation inhibitors which activity relies more on acting on de novo biosynthesis of GDP-fucose and/or other fucosyltransferases than FUT8, are stronger proliferation inhibitors.[16] The metabolic activity data suggests A2FF1P falls in this class of compounds along with Fucotrim I, Fucotrim II and Fucostatin I.[16,17,18]

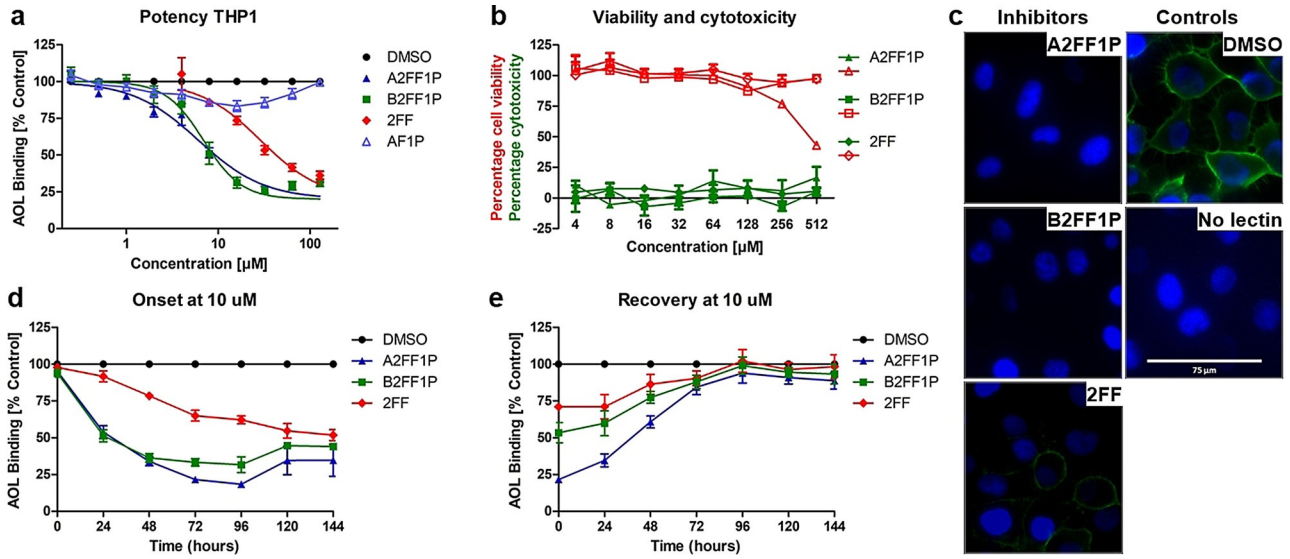


Figure 1. (a) A2FF1P, B2FF1P and 2FF inhibit fucosylation of THP1 cells. THP1 cells were treated with 0.25–128 μM fucose derivatives or DMSO control. After 3 days, the cells were stained with AOL lectin, followed by streptavidin-PE staining. Binding of lectins was determined by flow cytometry and data are presented as mean percentage lectin binding ± standard error of the mean (SEM) normalized to the control ($n=3$). The EC_{50} values were extrapolated for all compounds (Table 1). The level of fucosylation was determined with both the AOL and AAL lectin on THP1, HeLa and H1299 cells (Figure S1). (b) The effect on viability (red, open) and cytotoxicity (green, closed) on THP1 cells after incubation with 4–512 μM A2FF1P, B2FF1P and 2FF was determined with an XTT and LDH assay, respectively, and data presented as percentage cell viability or cytotoxicity compared to DMSO control. (c) Visualization of cellular defucosylation. H1299 cells were cultured for 3 days in the presence of 100 μM of A2FF1P, B2FF1P, 2FF or DMSO. The cells were then fixated, blocked and stained with fucose-recognizing biotinylated lectins AOL or AAL (Figure S2) and subsequently Streptavidin-AlexaFluor 488 (green). Nuclei were stained using NucBlue (blue). The white line in the 'No lectin' panel indicates the scale (75 μm) for all panels. (d–e) Onset and recovery of cell surface defucosylation. THP1 cells were incubated with 10 or 100 μM (Figure S3) A2FF1P, B2FF1P, 2FF or DMSO control and fucosylation levels were determined with AOL and AAL lectins (Figure S3) over the course of six days to determine the onset (d) or for six days after a 3 day incubation period and replacing the medium with fresh medium without inhibitors to determine the recovery (e).

Table 1. EC_{50} values in micromolar for inhibition of fucose expression.[a]

	THP-1		HeLa		H1299	
Compound	AAL	AOL	AAL	AOL	AAL	AOL
DMSO	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
A2FF1P (**6**)	6.38	6.11	55.8	44.0	29.7	24.8
B2FF1P (**5**)	7.68	6.94	54.2	41.4	40.2	34.5
2FF (**2**)	32.2	29.0	319	226	194	165

[a] Three cell lines (THP-1, HeLa and H1299) were cultured for 3 days with 0–128 μM A2FF1P or B2FF1P or 0–512 μM for 2FF (positive control) or DMSO (negative control). The cells were stained with two fucose specific lectins (AAL and AOL) and analyzed by flow cytometry. Data are presented as mean percentage lectin binding normalized to DMSO control ($n=3$). The EC_{50} values were determined for both lectins, defined as the concentration where a 50% decrease in lectin binding was observed. N.I. = no inhibition.

Inhibition of fucosylation by 2FF and the more potent A2FF1P and B2FF1P derivatives was also demonstrated by microscopy. H1299 cells were treated with 100 μM A2FF1P, B2FF1P, 2FF or DMSO for 3 days, after which the cells were stained with AOL (Figure 1c) or AAL (Figure S2) lectins. Specific labeling of membrane bound fucosylated glycans was observed in control cells treated with DMSO and staining was abolished in cells treated with A2FF1P or B2FF1P and to a lesser extent 2FF.

For therapeutic applications it is important to know more about the kinetics of A2FF1P, B2FF1P and 2FF in terms of onset and recovery of inhibition. Having established the increased potency of A2FF1P and B2FF1P over 2FF, the onset of defucosylation was determined by incubating THP-1 cells with 10 or 100 μM of the inhibitors and analyzed using the AAL and AOL lectins for six consecutive days (Figure 1d; Figure S3a–c). At a concentration of 10 μM cellular defucosylation was faster for the 1-phosphate derivatives, reaching 50 percent reduction after only one day. A small difference was observed for the α- and β-phosphates, suggesting they are metabolized similarly. At 100 μM concentration this difference in onset was less pronounced, possibly due to saturation of the salvage pathway enzymes with the unnatural fucose derivatives. Consequently, the turnover rate of membrane-bound fucosylated glycans may be a limiting step, thereby obscuring the potential differences in metabolic conversion of 2FF, B2FF1P and A2FF1P.

For assessing the recovery, cells were incubated with 10 or 100 μM of the inhibitors for three days, after which the inhibitors were removed from the culture medium, and fucose levels were measured for six consecutive days using the AAL and AOL lectins (Figure 1e; Figure S3d–f). Fucosylation levels after the initial three days of incubation ($T=0$ hours) with 10 μM differed for all three inhibitors, however fucosylation was recovered after three to four days for all three inhibitors. At 100 μM concentrations, the initial fucosylation levels were equally reduced for all three inhibitors and recovery to normal fucosylation levels was almost complete after six days. Again, these kinetic assays are dependent on expression of fucosylated glycans on the cell membrane and their turnover rates. This data

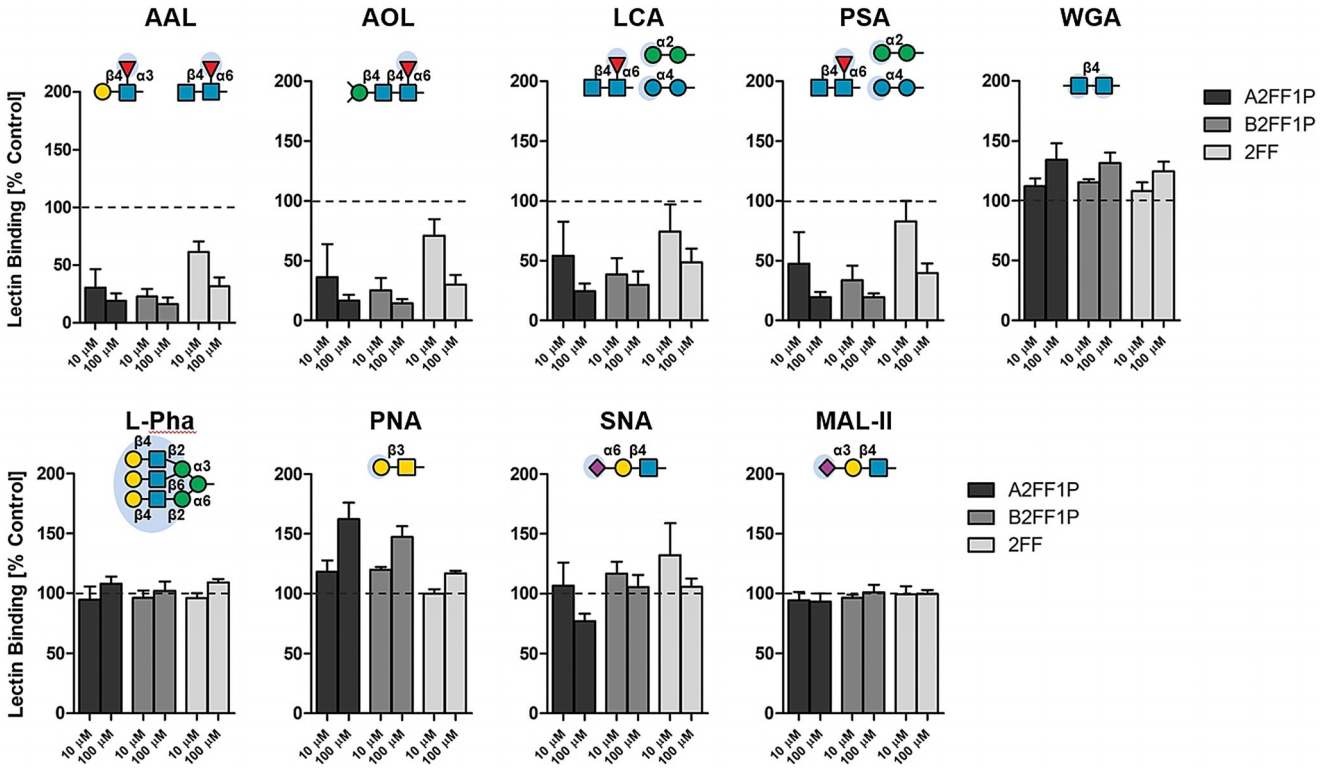


Figure 2. Lectin panel. THP1 cells were cultured for 3 days in the presence of 10 or 100 µM of A2FF1P, B2FF1P, 2FF or DMSO control and cell surface glycosylation was measured by flow cytometry using biotinylated lectins conjugated to streptavidin-PE. Presence of α-linked fucose (preferably 1,3- and 1,6-linked) was assessed with AAL, core fucose with AOL, α-mannose and α-glucose in combination with core fucose by both LCA and PSA, GlcNAc and sialic acids by WGA, complex *N*-glycans by L-Pha, exposure of terminal galactose by PNA, α2,6 sialylation by SNA and α2,3 sialylation by MAL-II. Data are presented as mean percentage lectin binding ± standard error of the mean (SEM) normalized to DMSO control (dotted line) ($n = 3$).

does show however that efficient, long-term inhibition can be achieved using these inhibitors.

Next, we evaluated the selectivity of the inhibitors for their effect on fucosylation in comparison to other glycans. To this end, THP1 cells were treated for three days with 10 or 100 µM A2FF1P, B2FF1P or 2FF and stained with a panel of lectins with distinct glycan specificities (Figure 2). We found that the fucose inhibitors specifically reduced the binding of fucose-recognizing lectins AAL, AOL, LCA, and PSA, but not of other glycan-recognizing lectins WGA, L-PHA, PNA, SNA and MAL-II. Notably, at 100 µM concentrations WGA and PNA binding was slightly increased for all three inhibitors, presumably due to better ligand binding upon fucose inhibition. These data demonstrate that A2FF1P, B2FF1P and 2FF specifically inhibit fucosylation without significantly affecting overall glycosylation.

Our results demonstrate that B2FF1P is considerably more potent than 2FF although both compounds share a similar mode of action, toxicity, viability, and selectivity profile. This is consistent with our hypothesis that feeding a more advanced metabolic precursor leads to more potent inhibition. On the other hand, it is surprising that A2FF1P is an equally potent inhibitor as B2FF1P since its anomeric configuration is reversed and to the best of our knowledge not found in cells. However, studies by Burkart et al. have shown that α-GDP-Fuc2F inhibited fucosyltransferases FucTV and FucTVI significantly in vitro, which could explain the inhibitory activity of A2FF1P if it is metabolized to its corresponding GDP-analogue in cells.[19]

To investigate the mechanism of action for B2FF1P and A2FF1P we performed nucleotide sugar analysis of THP1 cells treated with 50 µM A2FF1P or B2FF1P, 100 µM 2FF or DMSO control for different time points. After lysis, the intracellular nucleotide sugar levels were analyzed using reverse-phase ion pairing chromatography coupled to a triple quadrupole mass spectrometer operating in negative ion mode (Figure 3; Figure S4).[20] These experiments revealed a more efficient metabolism of B2FF1P towards its GDP-analogue over 2FF, even though two times higher concentrations were used for 2FF. This resulted in a higher intracellular concentration of β-GDP-Fuc2F. Surprisingly, we found that A2FF1P, bearing the unnatural anomeric configuration, was efficiently converted to another GDP-Fuc2F isomer than β-GDP-Fuc2F, eluting at 15.5 minutes (Figure 3a). Because this peak has a different retention time but a nearly identical fragmentation spectrum (Figure S5) and it is formed only in cells incubated with A2FF1P, we identified it as α-GDP-Fuc2F. Upon incubation with A2FF1P also an increase of β-GDP-Fuc2F was observed over time. Potentially, enzyme mediated or spontaneous hydrolysis of α-GDP-Fuc2F occurs forming an equilibrium α/β-mixture of 2-deoxy-2-fluorofucose which can be metabolized towards β-GDP-Fuc2F via a known mechanism (Scheme 1).[7]

Notably, an efficient depletion of the endogenous GDP-fucose pool was observed by incubation with A2FF1P and B2FF1P. Our data thus demonstrates that not only the binding domain of fucosyltransferase enzymes for the sugar moiety

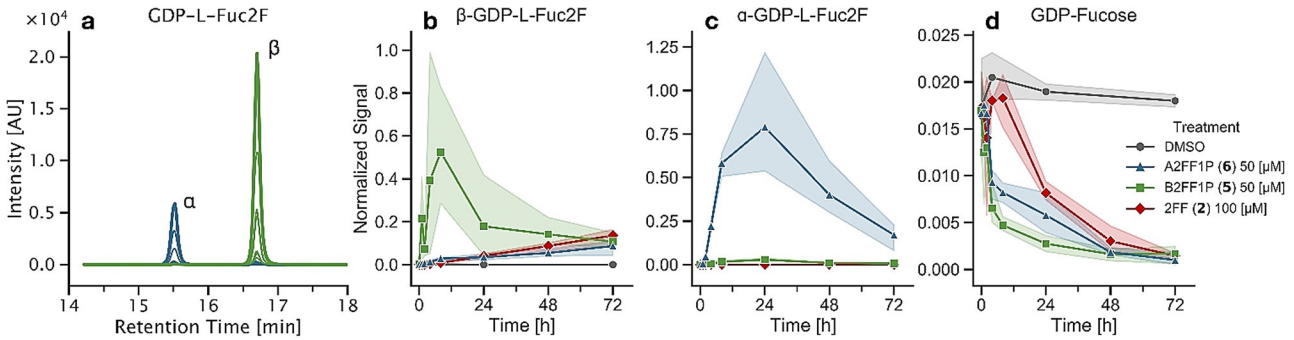


Figure 3. Nucleotide sugar analysis. THP1 cells were incubated for indicated time points with DMSO control, 50 μM A2FF1P, 50 μM B2FF1P or 100 μM 2FF. (a) Relative retention times of α- and β-GDP-L-Fuc2F by incubation with A2FF1P and B2FF1P for 4 hours. (b–d) After sample preparation, the β-GDP-L-Fuc2F (b), α-GDP-L-Fuc2F (c), β-GDP-L-Fucose (d) and other nucleotide levels (Figure S4) were analyzed using reverse-phase ion pairing chromatography coupled to a triple quadrupole mass spectrometer operating in negative ion mode and presented as their abundance in the nucleotide sugar pool ($n = 3$).

allows for stereochemical flexibility as previously reported but there is also promiscuity in GDP-fucose pyrophosphorylase activity that can be explored for further fucosylation inhibitor design.[19]

In conclusion, we developed two potent fucosylation inhibitors, A2FF1P and B2FF1P, based on fucose-1-phosphate derivatives. Their potency, specificity, duration of activity and low toxicity make them potential candidates for further therapeutic development. The finding that an α-fucose-1-phosphate analogue is metabolized towards its corresponding GDP-analogue raises the question which other modifications would be allowed and how these would affect activity. Moreover, it would be interesting to see if other (non-)endogenous glycosyl-1-phosphates share the same promiscuity in anomeric orientation for nucleotide sugar metabolism. If so, this could be the basis of a new type of inhibitors and tools for studying glycans.

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Conflict of interest

The authors declare no conflict of interest.

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[1] B. Ma, J. L. Simala-Grant, D. E. Taylor, *Glycobiology* **2006**, *16*, 158R–184R.
[2] K. T. Schjoldager, Y. Narimatsu, H. J. Joshi, H. Clausen, *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 729–749.
[3] M. Schneider, E. Al-Shareffi, R. S. Haltiwanger, *Glycobiology* **2017**, *27*, 601–618.
[4] J. Li, H.-C. Hsu, J. D. Mountz, J. G. Allen, *Cell Chem. Biol.* **2018**, *25*, 499–512.
[5] T. S. Keeley, S. Yang, E. Lau, *Cancers* **2019**, *11*, 1241.
[6] A. Blanas, N. M. Sahasrabudhe, E. Rodríguez, Y. van Kooyk, S. J. van Vliet, *Front. Oncol.* **2018**, *8*, 39.
[7] C. D. Rillahan, A. Antonopoulos, C. T. Lefort, R. Sonon, P. Azadi, K. Ley, A. Dell, S. M. Haslam, J. C. Paulson, *Nat. Chem. Biol.* **2012**, *8*, 661–668.
[8] Y. Zhou, T. Fukuda, Q. Hang, S. Hou, T. Isaji, A. Kameyama, J. Gu, *Sci. Rep.* **2017**, *7*, 11563.
[9] M. A. Carrascal, M. Silva, J. S. Ramalho, C. Pen, M. Martins, C. Pascoal, C. Amaral, I. Serrano, M. J. Oliveira, R. Sackstein, P. A. Videira, *Mol. Oncol.* **2018**, *12*, 579–593.
[10] N. M. Okeley, S. C. Alley, M. E. Anderson, T. E. Boursalian, P. J. Burke, K. M. Emmerton, S. C. Jeffrey, K. Klussman, C.-L. Law, D. Sussman, B. E. Toki, L. Westendorf, W. Zeng, X. Zhang, D. R. Benjamin, P. D. Senter, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 5404–5409.
[11] N. M. Okeley, R. A. Heiser, W. Zeng, S. M. Hengel, J. Wall, P. C. Haughney, T. A. Yap, F. Robert, R. E. Sanborn, H. Burris, L. Q. Chow, K. T. Do, M. Gutierrez, K. Reckamp, A. Weise, D. R. Camidge, J. Strickler, C. Steuer, Z. Wang, M. M. O'Meara, S. C. Alley, S. J. Gardai, *Cancer Res.* **2018**, *78*, 5551.
[12] M. L. Disis, L. R. Corulli, E. A. Gad, M. R. Koehnlein, D. L. Cecil, P. D. Senter, S. J. Gardai, N. M. Okeley, *Mol. Cancer Therapeutics* **2020**, *19*, 1102–1109.
[13] T. Heise, J. F. A. Pijnenborg, C. Büll, N. van Hilten, E. D. Kers-Rebel, N. Balneger, H. Elferink, G. J. Adema, T. J. Boltje, *J. Med. Chem.* **2019**, *62*, 1014–1021.
[14] E. M. Wright, D. D. F. Loo, B. A. Hirayama, *Physiol. Rev.* **2011**, *91*, 733–794.
[15] M. D. Burkart, Z. Zhang, S.-C. Hung, C.-H. Wong, *J. Am. Chem. Soc.* **1997**, *119*, 11743–11746.
[16] Y. Dai, R. Hartke, C. Li, Q. Yang, J. O. Liu, L.-X. Wang, *ACS Chem. Biol.* **2020**, *15*, 2662–2672.
[17] J. Pijnenborg, E. Rossing, M. Noga, W. Titulaer, R. Veizaj, D. J. Lefeber, T. Boltje, *ChemRxiv* **2020** https://doi.org/10.26434/chemrxiv.13082138.v1.
[18] J. G. Allen, M. Mujacic, M. J. Frohn, A. J. Pickrell, P. Kodama, D. Bagal, T. San Miguel, E. A. Sickmier, S. Osgood, A. Swietlow, V. Li, J. B. Jordan, K.-W. Kim, A.-M. C. Rousseau, Y.-J. Kim, S. Caille, M. Achmatowicz, O. Thiel, C. H. Fotsch, P. Reddy, J. D. McCarter, *ACS Chem. Biol.* **2016**, *11*, 2734–2743.
[19] M. D. Burkart, S. P. Vincent, A. Düffels, B. W. Murray, S. V. Ley, C.-H. Wong, *Bioorg. Med. Chem.* **2000**, *8*, 1937–1946.

[20] M. van Scherpenzeel, F. Conte, C. Büll, A. Ashikov, E. Hermans, A. Willems, W. van Tol, E. Kragt, E. E. Moret, T. Heise, J. D. Langereis, E. Rossing, M. Zimmermann, M. E. Rubio-Gozalbo, M. I. de Jonge, G. J. Adema, N. Zamboni, T. Boltje, D. J. Lefeber, *bioRxiv* **2020**, https://doi.org/10.1101/2020.09.15.288712.

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