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Glycomimetic affinity-enrichment proteomics identifies partners for a clinically-utilized iminosugar†

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Widescale evaluation of interacting partners for carbohydrates is an underexploited area. Probing of the 'glyco-interactome' has particular relevance given the lack of direct genetic control of glycoconjugate biosynthesis. Here we design, create and utilize a natural product-derived glycomimetic iminosugar probe in a Glycomimetic Affinity-enrichment Proteomics (Glyco-A^{e}P) strategy to elucidate key interactions directly from mammalian tissue. The binding partners discovered here and the associated genomic analysis implicate a subset of chaperone and junctional proteins as important in male fertility. Such repurposing of existing therapeutics thus creates direct routes to probing *in vivo* function. The success of this strategy suggests a general approach to discovering 'carbohydrate-active' partners in biology.

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Introduction

Approximately 2–3% of most genomes are devoted to carbohydrate-active proteins (CAP).[1] These include lectins that bind carbohydrates, glycosylhydrolases that degrade them and glycosyltransferases that construct them (http://www.cazy.org).[2] Deficiencies of CAP can lead to pathological states, such as congenital muscular dystrophies[3] and lysosomal storage disorders;[4] CAPs are implicated in a range of host–pathogen interactions that lead to disease.[5,6] Despite these vital roles, most current strategies for determining their interactions (*e.g.* arrays or assays), whilst powerfully allowing the determination of *in vitro* specificities, do not allow widescale probing of cellular or organismal samples. Affinity strategies have rarely been used to identify novel binding partners or profile the carbohydrate-active proteome.[7] One such conceivable strategy is to use clinically-utilized compounds to identify novel binding partners; this advantageously would allow the re-purposing of pre-approved drugs and hence facilitate rapid translation and application.

One glycomimetic suitable for this purpose is the iminosugar *n*-butyldeoxynojirimycin (*N*B-DNJ, FDA-approved in 2002 as Zavesca® (miglustat)). *N*B-DNJ is prescribed for the treatment of type 1 Gaucher disease, an inherited lysosomal storage disorder and Niemann–Pick type C disease;[8–10] in humans, *N*B-DNJ is generally well-tolerated. This powerful glycomimetic is therefore an archetype of modulation of glycobiology by small molecules. One of its most remarkable properties is that in certain mouse strains (from the C57-lineage,[11] *e.g.* C57BL/6, AKR/J and BALB/c) it induces reversible, dose-dependent male infertility at very low doses (15 mg per kg per day).[10,12] In contrast, other strains (of the Swiss Castle lineage, such as FVB/N[13]) display a phenotype insensitive to *N*B-DNJ-induced infertility.[12] Studies with C57BL/6·FVB/N interstrain hybrid mice have suggested multiple genes (and hence multiple protein targets) contribute to this striking function (infertility) induced by *N*B-DNJ.[12] This raises the intriguing possibility that modulation of the carbohydrate-active proteome may be intimately linked to reproduction. There are some early indications of the origins of this exciting effect. Treated mice display lower sperm counts and abnormal sperm morphologies (deformed or no acrosomes, non-falciform nuclei[10,12]) rendering them incapable of binding the zona pellucida to initiate fertilization.[14] However, the exact protein targets are unknown – providing a suitably challenging test of our strategy. *N*B-DNJ inhibits β-glucosidase 2 (GBA2), lysosomal acid β-glucosidase I (GBA) and glucosylceramide synthase (GCS/UGCG).[15–17] Knockout of GBA2 impairs mouse fertility and creates sperm abnormalities.[18] The epididymal spermatozoa of *N*B-DNJ-insensitive strains only

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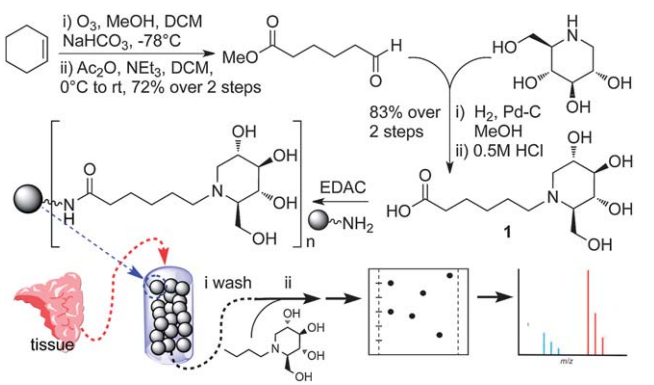
display minor morphological imperfections; accordingly, these mice are normally fertile.[12] However, all mouse strains show similar elevated level of glucosylceramide when treated with *N*B-DNJ. This suggests no direct link to glycosphingolipid metabolizing enzymes GBA/GBA2 and/or GCS/UGCG and implicates instead the differences in genetic background and other protein partners. In fact, to date, no comprehensive study of the cellular targets of *N*B-DNJ has been conducted and the protein(s) involved in induced male infertility remain unknown.

We report here a comprehensive affinity-enrichment proteomic (A^{e}P) study utilizing an immobilized glyco-affinity probe to identify proteins that interact with *N*B-DNJ and potentially responsible for its contraceptive activity. Immobilized iminosugars have previously been used for simple glycosidase affinity chromatography,[19–22] however, here we show in a proof-of-concept study how this archetypal glycomimetic can allow proteomics directed towards its interactome. Unlike designed purification methods, which intentionally exploit a known ligand-protein partnership for affinity, we choose *N*B-DNJ here as a clinically approved probe molecule that is known to induce phenotypic changes but in the absence of any such clear partnership(s). In this way, the 'glyco-A^{e}P' method has the potential to identify unforeseen protein–ligand interactions that may be important in a therapeutically-relevant phenotype and so, as we show here, reveals a relevant, previously not considered, focused subset of the carbohydrate-active proteome.

Result and discussion

A carboxyl-bearing probe ligand **1** derived from DNJ[23] was prepared in a two-step protecting-group-free synthesis employing methanolytic ozonolysis of cyclohexene[24] followed by reductive alkylation of DNJ (Scheme 1). The glyco-affinity probe **1** was immobilized on amino-terminated agarose support through 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC)-mediated amide bond formation (Scheme 1). The binding ability of the resulting glyco-A^{e}P probe matrix was validated with a clinically-utilized GBA preparation (ESI Fig. 1†);[16,25] this importantly confirmed functional utility through extraction of a known protein partner.

Next, tissue was collected from a mammal with relevant phenotype (mouse testis from *N*B-DNJ-sensitive male C57BL/6 mice) to identify proteins contributing to inducible infertility. Protein fraction from homogenized tissue was split and interrogated (see ESI†) with either glyco-A^{e}P probe matrix or unmodified agarose (control); washing removed unbound fractions. The many selected or enriched binding proteins were eluted from respective matrices with *N*B-DNJ-containing buffer and were concomitantly further fractionated and directly visualized by comparative 2D-polyacrylamide gel electrophoretic analysis (Scheme 1 and Fig. 1), detected by silver staining. These were directly analyzed by in-gel digestion and proteomic LC-MS/MS analysis. From 351 proteins identified, 64 that were also identified in the control were discounted from the glyco-A^{e}P screen (Fig. 2a). We applied the following strategy to minimize the chances of false-positive identification of contaminant proteins: Only significant (p-value cut-off < 0.05) protein identifications were accepted throughout all searches. Protein hits in the enriched sample were only considered, if identified twice (in replicates 1 and 2). At the same time all protein hits which were found in either replicate of the control sample (replicate 1 or 2) were discarded and not considered as potential interacting proteins (see ESI and later for additional discussion†). This generated a focused list of 18 proteins reliably identified through glyco-A^{e}P probing (but not in controls) as strong carbohydrate-active candidates. Cross-validation of theoretical masses and isoelectric points allowed further narrowing of this focus and reduced the protein cohort to six (Table 1) with plausible functional roles.



Scheme 1 Glyco-A^{e}P probe matrix and method.

Two Hsp70 proteins (HSPA2 & Hypoxia up-regulated protein 1 (HYOU1)) were identified. The testis functions on the brink of hypoxia;[26] low oxygen levels in the testis are required for spermatogenesis.[27] Proteins that are upregulated by low oxygen concentrations, such as HYOU1 (Grp170), may play key roles in spermatogenesis. Furthermore, HYOU1 directly regulates (Fig. 2b) insulin (INS)[28] and vascular endothelial growth factor (VEGF).[29] Abnormal VEGF levels in seminal plasma correlate with IVF pregnancy success.[30] INS affects reproductive function in humans and animals at multiple levels by effecting endocrine control of spermatogenesis, as well as on mature ejaculated spermatozoa.[31] The second Hsp70 protein, HSPA2, is a testis-specific form in mice, where it is regulated developmentally and expressed in spermatogenic cells.[32] It has a unique role during germ cell differentiation[33] and is necessary for progression of meiosis in mouse germ cells.[32]

Junction plakoglobin (JUP, desmoplakin 3) is a junctional plaque protein involved in the formation of desmosomes and tight junctions.[34] Mice with impaired ability to form tight junctions (*e.g.* Epas1$^{-/-}$) display higher testicular oxygen levels, which interferes with spermatogenesis.[27] It is conceivable that desmosome disruption by *N*B-DNJ may affect spermatogenesis by such a mechanism. Indeed reversible male infertility has been demonstrated by unrelated small molecules that disrupt adherens junctions in the testis.[35]

Intriguingly, we have discovered here the protein SET (also known as template activating factor 1β, a histone chaperone for nucleosome assembly[36] and as I2PP2A[37]). I2PP2A inhibits multifunctional protein phosphatase PP2A. Notably, I2PP2A binds sphingolipids, including ceramide and PTY720, a clinical

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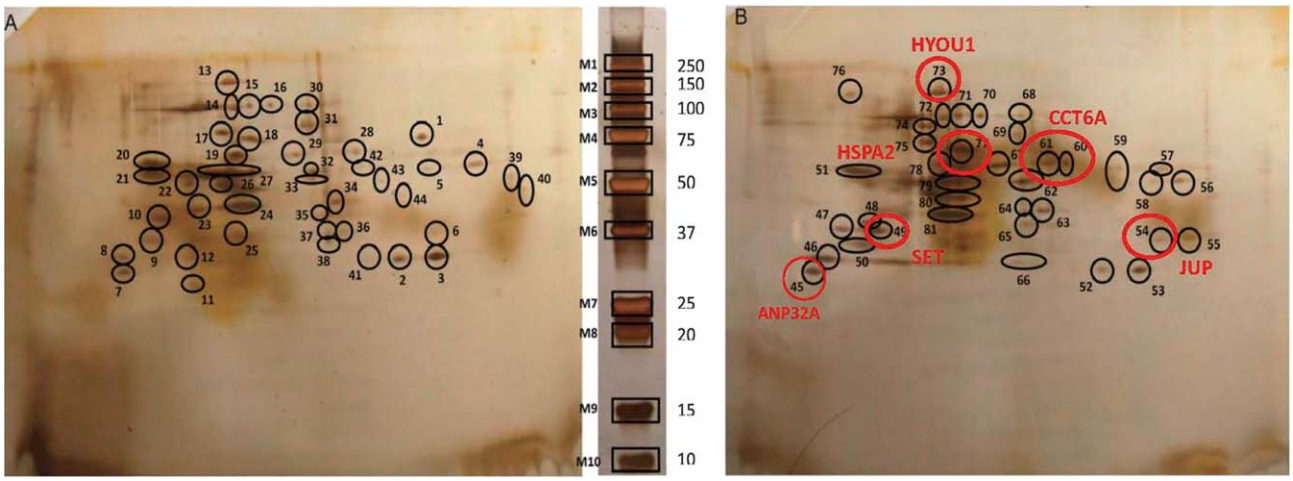

Fig. 1 Comparative 2D-polyacrylamide gel electrophoretic analysis of C57BL/6 mouse testis tissue interrogated with unmodified control (panel A) and glycomimetic affinity (panel B) matrices. pI scale 3–10. Middle: molecular weight marker.

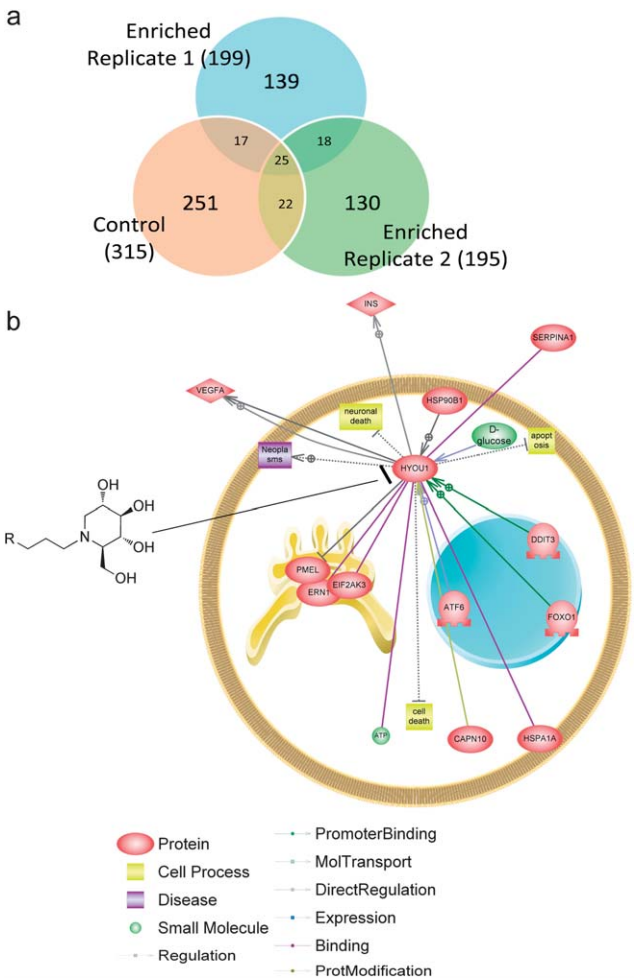

Fig. 2 (a) Venn analysis of glyco-A^{e}P proteins identified (here at score > 20 – see ESI for other score Venn analyses†). (b) Pathway analysis of primary glyco-A^{e}P-identified target HYOU1 (Pathway Studio 9). HYOU1 is influenced by Glc and directly regulates VEGFA & INS as possible drivers of temporary male infertility.

sphingosine analogue.[38] Given that miglustat, in its inhibition of GCS, competitively inhibits ceramide binding, it may be miglustat binds SET/I2PP2A in a similar manner.[39]

Interestingly, several of the cohort proteins are chaperones: not only HYOU1, HSPA2, and SET but also T-complex protein 1 subunit zeta (CCT6A), a chaperone protein involved in the folding of tubulin and actin and other proteins.[40] Notably, *N*B-DNJ sensitive strains (C57BL/6) show decreased levels of acrosomal proteins, after treatment with *N*B-DNJ, compared with non-sensitive strains (FVB/N).[12] It is possible that *N*B-DNJ impairment of chaperone protein function may be responsible for this phenotype. Some of the selectively identified partners have no obvious potential role. The acidic leucine-rich nuclear phosphoprotein 32 family members (ANP32) (spot 45) have been implicated in a number of cellular processes including cell cycle progression, differentiation and apoptosis.[41] However, gene disruption studies of ANP32A produced mouse strains which were both viable and fertile[42] suggesting that these proteins are not directly implicated in *N*B-DNJ induced male infertility and potentially highlight a functionally unrelated interaction.

Together these data implicate a glycomimetic-interactome (Hsps, junctional proteins, chaperones and ceramide binders) that would not have been readily predicted but that now suggest functionally plausible pathways (*e.g.*, the effects of chaperones and tight junction assembly on spermiogenesis) for investigation that could lead to validated drug targets for contraception. Inspection of the genetic differences that code for this proteome between sensitive and insensitive mice allows identification of single-nucleotide polymorphisms (SNPs). SNP analysis (see ESI for further details†) was applied to genes encoding for the proteins identified using the glyco-A^{e}P strategy examining differences between miglustat-sensitive strain C57BL/6 and insensitive strain FVB/N.[13] Annotated gene sequences were searched 5 kb both upstream and downstream. No SNPs were found for *Hyou1*, *Jup*, *Anp32b* or *Set* but were for *Cct6a* (180), *Hspa2* (57 total) and *Anp32a* (367) (see ESI†). In *Cct6a*

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Table 1 Significant proteins identified from mouse testis with putative *N*B-DNJ affinities using glyco-A^{e}P[b]

Spot	Accession number	Protein name	Code	Score	M_r (Da)	pI	% Coverage
73[a]	Q9JKR6	Hypoxia up-regulated protein 1	HYOU1	291	111 340	5.12	68
54	Q02257	Junction plakoglobin	JUP	65	82 490	5.75	37.3
77	P17156	Heat shock-related 70 kDa protein 2	HSPA2	92	69 884	5.51	51
45	O35381	Acidic leucine-rich nuclear phosphoprotein 32 family member A	ANP32A	86	28 691	3.99	23.9
49	Q9EQU5	Protein SET	SET	49	33 358	4.22	29.1
60/61	P80317	T-complex protein 1 subunit zeta	CCT6A	45	58 424	6.63	51.8

[a] Bold italic indicates proteins unique to the enriched sample; bold indicates enriched. [b] Membrane-bound CAP glycosidases & glycosyltransferases require bespoke separation methods (*e.g.* sucrose gradient of golgi membranes)[7] and this may lead to under-representation in glyco-A^{e}P.

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(NM_009838) the majority of the SNPs were found in non-coding regions including the 3′ untranslated region (3′UTR). However, six changes were found in the coding region: five of which do not change the corresponding amino acid. One in exon 9, Chr5 position 130299586 has the SNP designation rs13470985 and changes amino acid 348 from E (C57BL/6) to G in (FVB/N). In *Hspa2* (NM_001002012) again non-coding region SNPs were the majority (including the 3′UTR); three SNPs were identified in the coding region but these do not change the corresponding amino acids. In *Anp32* (NM_009672) only SNPs in non-coding regions (including 3′UTR) were found. As well as the single CCT6A-E348G difference identified, it cannot be discounted that the identified non-coding changes may affect gene regulation. However, in the absence of microarray analysis of FVB/N testis tissue it is not yet possible to compare expression levels.

It should be noted that there might be inherent variability in tissue sample, phenotypic state and through handling variations. However, it is noteworthy that the method showed apparent robustness in this regard; an additional glyco-A^{e}P experimental round using testis tissue successfully identified with high significance five out of the six proteins identified in the primary rounds (see ESI†). Preliminary experiments using brain tissue did not identify these proteins.

In addition, the methods of data analysis, performed here using the MOWSE scoring system as implemented by MASCOT (see ESI†)[43] will have a profound effect upon initial agreements on identified proteins (see the ESI† for extended discussion and analysis of the effect of scoring thresholds). Notably, at low thresholds (Fig. 2a, score > 20) there is lower initial overlap between enriched sets, whereas at higher thresholds (ESI, >50†) there is almost complete overlap (all non-excluded proteins in set 2 are also found in set 1). Notably all of the proteins listed in Table 1 have a score significantly above the threshold of 33 (>45, in fact) that has been recommended for mammalian tissue samples.[44]

Conclusions

In summary, the repurposing of an existing therapeutic is a potentially strong strategy for probes with wide-ranging utility, since it creates a more direct link to *in vivo* function. Although strategies in some key areas of biology have followed this logic,[45–48] the detailed examination of the modulation of glyco-biological function has, until now, not examined such valuable interactomes. Here using a glyco-A^{e}P strategy that exploits the archetypal iminosugar mimetic and therapeutic *N*B-DNJ/miglustat/Zavesca we have elucidated a focused subsection of the proteome hypothetically relevant to mammalian reproduction.

These data suggest new interactions (Hsps, junctional proteins, chaperones, ceramide binders) that would not have been readily predicted and that might help define the mechanisms by which *N*B-DNJ causes male infertility. Notably, the primary probe used here (**1**) is a derivative of the natural product nojirimycin.[49] The increasing utility of both functionalized natural products[50] and therapeutics, as two classes of small molecules that have been selected in contrasting manners for protein interaction, suggests that future affinity strategies might also be usefully based on their exploitation. To this end we are currently exploring extension of the glyco-A^{e}P strategy to other small molecule, natural product glycomimetics.

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References

1 P. M. Coutinho, E. Deleury, G. J. Davies and B. Henrissat, *J. Mol. Biol.*, 2003, **328**, 307.
2 B. L. Cantarel, P. M. Coutinho, C. Rancurel, T. Bernard, V. Lombard and B. Henrissat, *Nucleic Acids Res.*, 2009, **37**, D233.
3 D. J. Blake, A. Weir, S. E. Newey and K. E. Davies, *Physiol. Rev.*, 2002, **82**, 291.
4 K. Tetsuya and N. Manabu, *Curr. Top. Med. Chem.*, 2009, **9**, 13–33.
5 L. Gattegno, A. Ramdani, T. Jouault, L. Saffar and J. Gluckman, *AIDS Res. Hum. Retroviruses*, 1992, **8**, 27.

Published on 08 July 2013. Downloaded by University of Oxford on 29/07/2013 16:37:26.

6 R. Kannagi, M. Izawa, T. Kioke, K. Miyazaki and N. Kimura, *Cancer Sci.*, 2004, **95**, 377.
7 C.-H. Lin, C.-W. Lin and K.-H. Khoo, *Proteomics*, 2008, **8**, 475.
8 J. M. F. G. Aerts, W. E. Donkerkoopman, M. K. Vandervliet, L. M. V. Jonsson, E. I. Ginns, G. J. Murray, J. A. Barranger, J. M. Tager and A. W. Schram, *Eur. J. Biochem.*, 1985, **150**, 565.
9 J. M. F. G. Aerts, W. E. Donkerkoopman, M. Koot, J. A. Barranger, J. M. Tager and A. W. Schram, *Clin. Chim. Acta*, 1986, **158**, 155.
10 A. C. van der Spoel, M. Jeyakumar, T. D. Butters, H. M. Charlton, H. D. Moore, R. A. Dwek and F. M. Platt, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 17173.
11 J. A. Beck, S. Lloyd, M. Hafezparast, M. Lennon-Pierce, J. T. Eppig, M. F. Festing and E. M. Fisher, *Nat. Genet.*, 2000, **24**, 23.
12 W. Bone, C. M. Walden, M. Fritsch, U. Voigtmann, E. Leifke, U. Gottwald, S. Boomkamp, F. M. Platt and A. C. van der Spoel, *Reprod. Biol. Endocrinol.*, 2007, **5**, 1.
13 J. R. Wang, F. P.-M. deVillena, H. A. Lawson, J. M. Cheverud, G. A. Churchill and L. McMillan, *Genetics*, 2012, **190**, 449.
14 R. Suganuma, C. M. Walden, T. D. Butters, F. M. Platt, R. A. Dwek, R. Yanagimachi and A. C. van der Spoel, *Biol. Reprod.*, 2005, **72**, 805.
15 H. Q. Li, T. Liu, Y. M. Zhang, S. Favre, C. Bello, P. Vogel, T. D. Butters, N. G. Oikonomakos, J. Marrot and Y. Bleriot, *ChemBioChem*, 2008, **9**, 253.
16 F. M. Platt, G. R. Neises, R. A. Dwek and T. D. Butters, *J. Biol. Chem.*, 1994, **269**, 8362.
17 C. M. Walden, R. Sandhoff, C. C. Chuang, Y. Yildiz, T. D. Butters, R. A. Dwek, F. M. Platt and A. C. van der Spoel, *J. Biol. Chem.*, 2007, **282**, 32655.
18 Y. Yildiz, H. Matern, B. Thompson, J. C. Allegood, R. L. Warren, D. M. O. Ramirez, R. E. Hammer, F. K. Hamra, S. Matern and D. W. Russell, *J. Clin. Invest.*, 2006, **116**, 2985.
19 C. Bernotas and B. Ganem, *Biochem. J.*, 1990, **270**, 539.
20 A. Faridmoayer and C. H. Scaman, *Protein Expression Purif.*, 2004, **33**, 11.
21 H. Matern, H. Heinemann, G. Legler and S. Matern, *J. Biol. Chem.*, 1997, **272**, 11261.
22 P. Scudder, D. C. A. Neville, T. D. Butters, G. W. J. Fleet, R. A. Dwek, T. W. Rademacher and G. S. Jacob, *J. Biol. Chem.*, 1990, **265**, 16472.
23 H. Paulsen, I. Sangster and K. Heyns, *Chem. Ber.*, 1967, **100**, 802.
24 S. L. Schreiber, R. E. Claus and J. Reagan, *Tetrahedron Lett.*, 1982, **23**, 3867.
25 P. Alfonso, S. Pampin, J. Estrada, J. C. Rodriguez-Rey, P. Giraldo, J. Sancho and M. Pocovi, *Blood Cells, Mol., Dis.*, 2005, **35**, 268.
26 J. J. Lysiak, Q. A. T. Nguyen and T. T. Turner, *Biol. Reprod.*, 2000, **63**, 1383.
27 M. Gruber, L. K. Mathew, A. C. Runge, J. A. Garcia and M. C. Simon, *Biol. Reprod.*, 2010, **82**, 1227.
28 T. Kobayashi and Y. Ohta, *Pancreas*, 2005, **30**, 299.
29 G. L. Semenza, *J. Clin. Invest.*, 2001, **108**, 39.
30 A. Obermair, A. Obruca and M. Pohl, *Fertil. Steril.*, 1999, **72**, 269.
31 F. Lampiao, A. Agarwal and S. S. du Plessis, *Arch. Med. Sci.*, 2009, **5**, S48.
32 E. M. Eddy, *Rev. Reprod.*, 1999, **4**, 23.
33 M. J. Vos, J. Hageman, S. Carra and H. H. Kampinga, *Biochemistry*, 2008, **47**, 7001.
34 A. P. Kowalczyk, P. Navarro, E. Dejana, E. A. Bornslaeger, K. J. Green, D. S. Kopp and J. E. Borgwardt, *J. Cell Sci.*, 1999, **111**, 3045.
35 D. D. Mruk, C-H. Wong, B. Silverstrini and C. Y. Cheng, *Nat. Med.*, 2006, **12**, 1323.
36 K. Kato, M. Okuwaki and K. Nagata, *J. Cell Sci.*, 2011, **124**, 3254.
37 M. Li, A. Makkinje and Z. Damuni, *J. Biol. Chem.*, 1996, **271**, 11059.
38 S. A. Saddoughi, S. Gencer, Y. K. Peterson, K. E. Ward, A. Mukhopadhyay, J. Oaks, J. Bielawski, Z. M. Szulc, R. J. Thomas, S. P. Selvam, C. E. Senkal, E. Garrett-Mayer, R. M. De Palma, D. Fedarovich, A. Liu, A. A. Habib, R. V. Stahelin, D. Perrotti and B. Ogretmen, *EMBO Mol. Med.*, 2013, **5**, 105.
39 T. D. Butters, L. A. G. M. van den Broek, G. W. J. Fleet, T. M. Krulle, M. R. Wormald, R. A. Dwek and F. M. Platt, *Tetrahedron: Asymmetry*, 2000, **11**, 113.
40 Y. J. Gao, J. O. Thomas, R. L. Chow, G. H. Lee and N. J. Cowan, *Cell*, 1992, **69**, 1043.
41 P. T. Reillya, S. Afzal, C. Gorrini, K. Lui, Y. V. Bukhman, A. Wakeham, J. Haight, T. W. Ling, C. C. Cheung, A. J. Elia, P. V. Turner and T. W. Mak, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 10243.
42 P. Opal, J. J. Garcia, A. E. McCall, B. S. Xu, E. J. Weeber, J. D. Sweatt, H. T. Orr and H. Y. Zoghbi, *Mol. Cell. Biol.*, 2004, **24**, 3140.
43 D. N. Perkins, D. J. Pappin, D. M. Creasy and J. S. Cottrell, *Electrophoresis*, 1999, **20**, 3551.
44 T. Koenig, B. H. Menze, M. Kirchner, F. Monigatti, K. C. Parker, T. Patterson, J. J. Steen, F. A. Hamprecht and H. Steen, *J. Proteome Res.*, 2008, **7**, 3708.
45 V. E. Albrow, C. Fernandes, D. M. Beal, M. D. Selby, M. Fernandez-Ocana, K. C. Rumpel and L. H. Jones, *Med. Chem. Commun.*, 2012, **3**, 322.
46 T. T. Aye, S. Mohammed, H. W. P. van den Toorn, T. A. B. van Veen, M. A. G. van der Heyden, A. Scholten and A. J. R. Heck, *Mol. Cell. Proteomics*, 2009, **8**, 1016.
47 P. Dadvar, M. O'Flaherty, A. Scholten, K. Rumpel and A. J. R. Heck, *Mol. BioSyst.*, 2009, **5**, 472.
48 S. E. Ong, M. Schenone, A. A. Margolin, X. Y. Li, K. Do, M. K. Doud, D. R. Mani, L. Kuai, X. Wang, J. L. Wood, N. J. Tolliday, A. N. Koehler, L. A. Marcaurelle, T. R. Golub, R. J. Gould, S. L. Schreiber and S. A. Carr, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 4617.
49 S. Inouye, T. Tsuruoka and T. Niida, *J. Antibiot.*, 1966, **19**, 288.
50 N. S. Hegde, D. A. Sanders, R. Rodriguez and S. Balasubramanian, *Nat. Chem.*, 2011, **3**, 829.

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