Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2024, **22**, 9403

Non-natural sialic acid derivatives with *o*-nitrobenzyl alcohol substituents for light-mediated protein conjugation and cell imaging†

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Received 25th September 2024,
Accepted 24th October 2024

DOI: 10.1039/d4ob01563k

rsc.li/obc

We have synthesized two sialic acid derivatives substituted with an *ortho*-nitrobenzyl alcohol (***o*-NBA**) group that can undergo light-mediated conjugation with primary amines at their 5- or 9-carbon position. The ***o*-NBA** derivatives were shown to react with multiple lysine residues of human serum albumin (HSA) when exposed to 365 nm light irradiation within 10 min. The resulting sugar conjugates were characterized by mass spectroscopy and used for fluorescence-based cell imaging.

Glycosylation is an important post-translational modification (PTM) of proteins.[1,2] Protein glycosylation plays an essential role in cell physiology mediating immune responses, cell adhesion, cell–cell interactions and signal transduction.[3] Abnormal glycosylation levels is a hallmark for health related conditions including inflammation, autoimmune disorders, neurodegenerative diseases and cancer.[4–7] Sialic acids are a common terminal sugar of N- and O-linked glycans. Sialylation is abnormally upregulated in a diverse range of cancers including breast,[8] pancreatic,[9] ovarian,[10] brain,[11] lung,[12] prostate,[13] colorectal,[14] renal,[15] skin[16] and liver cancer.[17]

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†Electronic supplementary information (ESI) available: Experimental section, additional figures and original spectral copies of new compounds. See DOI: https://doi.org/10.1039/d4ob01563k

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In order to detect glycosylation in live cells, non-natural sugar probes have been developed. A general strategy is to install biorthogonal handles (such as an azide) to the side chain of a monosaccharide without perturbing its natural metabolic activity.[18–20] Then after incubation with live cells, a biorthogonal unit (such as an alkyne) linked with a fluorophore is added to react with the metabolically processed sugar probe, thus enabling the spatiotemporal monitoring of biomolecular glycosylation.[21,22] This elegant strategy has not only significantly advanced glycobiology, but provided insight into carbohydrate-based drug discovery.[23,24] However, while sugar probes for metabolic labelling of glycans have been extensively reported, those capable of capturing sugar-binding proteins remain much less explored.

To achieve the *in situ* capture of proteins, a reactive handle needs to be introduced into the biomolecules for biorthogonal conjugation. The prototypical reactive units commonly used include benzophenone, diazirine, aryl azide, tetrazole, and thienyl-substituted α-ketoamide.[25] Recently developed photocatalytic biorthogonal reactions are particularly advantageous for live cell imaging because of their biocompatibility and the ability to achieve biomolecular labelling in an on-demand fashion.[26] Recently, Chen *et al.* developed a strategy using *ortho*-nitrobenzyl alcohol (***o*-NBA**) as a light-activatable reactive handle to modify nucleic acids, and the resulting non-natural biomolecular probes were shown to sensitively capture nucleic acid-binding proteins.[27] This is because the nitroso benzaldehyde intermediate generated by UV light irradiation can undergo selective cyclization with primary amines of proteins.[28–31] This prompted us to explore the use of ***o*-NBA** as a reactive handle to modify sugars for light-mediated protein conjugation.

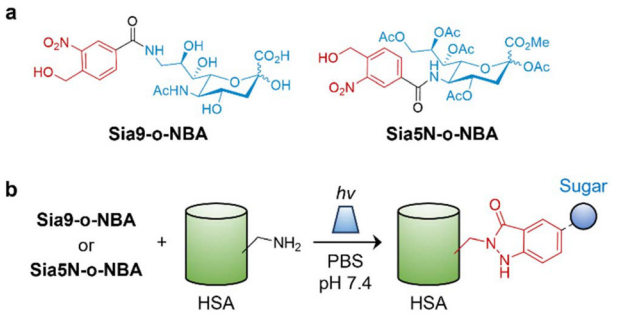


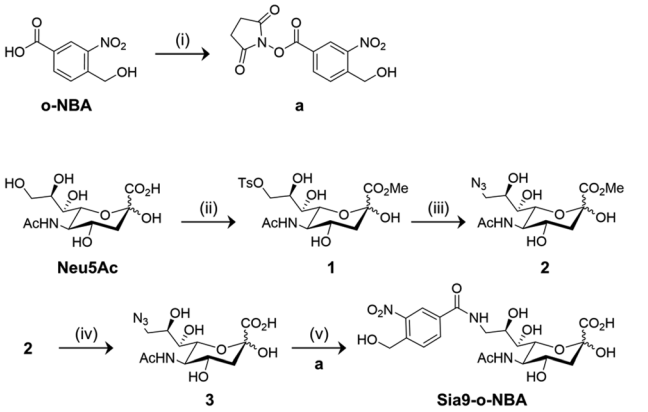
Fig. 1 (a) Structure of the ***o*-NBA**-modified sialic acid derivatives for (b) light-mediated covalent conjugation with human serum albumin (HSA).

Here, we designed and synthesized two sialic acid derivatives modified with an ***o*-NBA** group attached to the 5- or 9-postion capable of light-mediated coupling with primary amines (Fig. 1a). As a proof-of-concept, the resulting sugar probes were used for light-catalyzed conjugation with human serum albumin (HSA) in which lysine residues (with amino side chain) are abundant (Fig. 1b). The resulting sugar–HSA conjugates were characterized by mass spectroscopy and were used for cell imaging using an encapsulated fluorescent imaging agent.

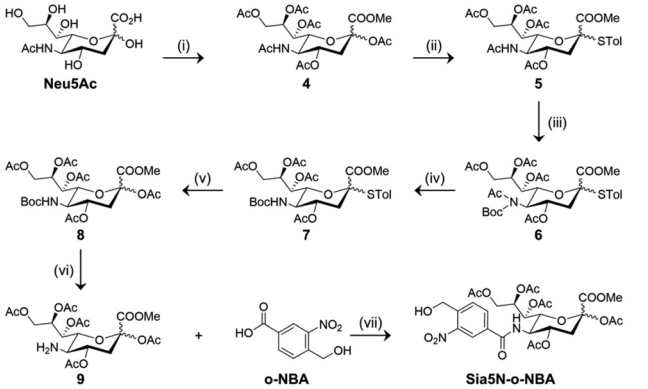
C-2 substituted peracetylated *N*-acetyl mannosamine (ManNAc) has been used as a non-natural sugar probe to detect cell sialylation after undergoing a series of biochemical transformations.[23] However, introducing bulky functional groups to the C-2 position of ManNAc may compromise substrate recognition by endogenous enzymes that mediate sialylation.[32] To address this issue, non-natural sialic acid derivatives are being developed to minimize perturbation of the subsequent sialylation biochemical pathways.[33,34] According to previous literature reports, we synthesized the key sialic acid intermediates **3** and **9**,[35–37] and the subsequent introduction of an ***o*-NBA** group by an amidation reaction allowed for the 9- (**Sia9-o-NBA**) and 5-substituted (**Sia5N-o-NBA**) non-natural sialic acid derivatives to be successfully prepared, respectively (Schemes 1 and 2).

Sialic acid intermediate **3** was obtained according to previous literature methods.[35,36] Briefly, the commercially available *N*-acetylneuric acid (**Neu5Ac**) was transformed into its methyl ester in the presence of Dowex 50WX2 H^+ resin to protect the carboxyl acid group. The hydroxyl group at the C9 position of this intermediate was then treated with tosyl chloride in pyridine to obtain the tosylated derivative **1**. Azide **3** was obtained through the reaction of sodium azide with **1** in methanol to afford intermediate **2**, followed by saponification with LiOH. Finally, **3** was reduced with Pd/C in water to yield an amine intermediate, followed by amidation with *N*-hydroxysuccinimide-activated ***o*-NBA** (**a**) to give **Sia9-o-NBA** in 15% yield (Scheme 1).

Likewise, intermediate **9** was obtained according to a previous literature report.[37] Starting from **Neu5Ac**, esterification and then per-acetylation afforded a fully protected sugar product **4**. Treatment of **4** with *p*-thiocresol (TolSH) in the presence of $BF_3{\cdot}Et_2O$ as the Lewis acid catalyst for 24 h afforded thioglycoside **5**. In order to selectively remove the C5 *N*-acetyl group, an additional *t*-butyloxycarbonyl (Boc) group was introduced to protect the secondary amine, affording intermediate **6**. Subsequently, deacetylation in a mixture of MeONa/MeOH followed by acetylation with Ac_2O and 4-dimethylaminopyridine (DMAP) gave the C5 Boc-protected sugar **7**. A short (2 h) treatment of **7** with *N*-bromosuccinimide (NBS) in a mixture of acetone/water set free the C1 alcohol, which was immediately acetylated with Ac_2O/DMAP to give **8**. The Boc group of **8** was selectively removed with trifluoroacetic acid to obtain amine **9**, which underwent amidation with ***o*-NBA** in



Scheme 1 Synthesis of **Sia9-o-NBA**. Conditions and reagents: (i) *N*-hydroxy succinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride·HCl (EDC·HCl) at room temperature (rt) for 2 h. (ii) Dowex 50WX2 H^+ resin in MeOH at 60 °C for 2 h, and then tosyl chloride in pyridine from 0 °C to rt overnight. (iii) NaN_3 in MeOH at reflux for 24 h. (iv) LiOH in MeOH/H_2O at rt for 2 h. (v) H_2, Pd/C at rt overnight, and then **a** in triethylamine at rt overnight.



Scheme 2 Synthesis of **Sia5N-o-NBA**. Conditions and reagents: (i) Dowex 50WX2 H^+ resin in MeOH at 60 °C for 2 h, and then Ac_2O and DMAP in pyridine at rt for 2 h. (ii) *p*-Thiocresol and $BF_3{\cdot}Et_2O$ in CH_2Cl_2 at rt overnight. (iii) Boc_2O and DMAP in THF at 60 °C for 8 h. (iv) MeONa/MeOH at rt for 2 h, and then Ac_2O and DMAP in pyridine at rt for 2 h. (v) *N*-Bromosuccinimide in acetone/H_2O at rt for 2 h, and then Ac_2O and DMAP in pyridine at rt for 2 h. (vi) Trifluoroacetic acid in CH_2Cl_2 at rt for 2 h. (vii) 2-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate and diisopropylethylamine in CH_2Cl_2 overnight.

the presence of 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethylamine (DIPEA) to produce **Sia5N-o-NBA** in 18% yield (Scheme 2).

With the non-natural sialic acid derivatives in hand, we examined their capacity for light-mediated protein conjugation using HSA as a model. The protecting group-free **Sia9-o-NBA** was used for the study. To a phosphate buffered saline (PBS, 0.01 M, pH 7.4) solution of HSA, an excess of **Sia9-o-NBA** was added. The resulting mixture was exposed to 365 nm light irradiation for 10 min, and then incubated at 37 °C for another 30 min. Subsequently, the unreacted sialic acid derivatives were removed through dialysis to afford the **Sia9-o-NBA-HSA** conjugates, which were then digested with trypsin for LC-MS/MS analysis. The results showed that 20 lysine sites of HSA were modified with **Sia9-o-NBA** after light irradiation (Fig. 2). These results indicate the effectiveness of the sialic acid derivative for light-mediated protein conjugation.

Given that sialic acid-binding proteins are commonly detected in a variety of mammalian cells, we attempted to use the sialic acid–HSA conjugates for fluorescence-based cell imaging. Chlorin e6 (**Ce6**), which is known to bind HSA in its IB domain, was used as the fluorescent imaging agent.[38] HSA without and with conjugation with **Sia9-o-NBA** were incubated with **Ce6** for 6 h, followed by dialysis to obtain **Ce6**-encapsulated HSA derivatives **Ce6@HSA** and **Ce6@Sia9-o-NBA-HSA**. Fluorescence spectroscopy indicated the presence of the typical **Ce6** emission band for all protein conjugates when dissolved in PBS solution (Fig. S1†).

RAW264.7 (mouse macrophage) and HeLa (human cervical cancer) cells were used for the imaging experiments. We observed that the HSA-based imaging agents were internalized by both cell lines after incubation for 1 hour at a **Ce6** concentration of 5 μM (Fig. 3a). The fluorescence intensity of the acquired images was then quantified using a Columbus™ Image Data Storage and Analysis System (Fig. 3b). Quantitative analysis revealed a higher cellular uptake of **Ce6@Sia9-o-NBA-HSA** by RAW264.7 than HeLa cells. This difference is probably caused by a higher Siglec (a family of sialic acid-binding proteins) expression level in immune cells.[39] Indeed, a subsequent analysis by quantitative real-time polymerase chain reaction corroborated the significantly higher expression level of several Siglec subtypes in RAW264.7 than HeLa cells (Fig. 3c). As a result, our sialic acid-conjugated HSA could be used to target macrophages for a diverse range of biomedical applications.

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  1 MKWVTFISLL FLFSSAYSRG VFRRDAHKSE VAHRFKDLGE ENFKALVLIA
 51 FAQYLQQCPF EDHVKLVNEV TEFAKTCVAD ESAENCDKSL HTLFGDKLCT
101 VATLRETYGE MADCCAKQEP ERNECFLQHK DDNPNLPRLV RPEVDVMCTA
151 FHDNEETFLK KYLYEIARRH PYFYAPELLF FAKRYKAAFT ECCQAADKAA
201 CLLPKLDELR DEGKASSAKQ RLKCASLQKF GERAFKAWAV ARLSQRFPKA
251 EFAEVSKLVT DLTKVHTECC HGDLLECADD RADLAKYICE NQDSISSKLK
301 ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADFVESKDVC KNYAEAKDVF
351 LGMFLYEYAR RHPDYSVVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE
401 FKPLVEEPQN LIKQNCELFE QLGEYKFQNA LLVRYTKKVP QVSTPTLVEV
451 SRNLGKVGSK CCKHPEAKRM PCAEDYLSVV LNQLCVLHEK TPVSDRVTKC
501 CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL SEKERQIKKQ
551 TALVELVKHK PKATKEQLKA VMDDFAAFVE KCCKADDKET CFAEEGKKLV
601 AASQAALGL
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Fig. 2 Analysis of HSA modified with **Sia9-o-NBA** by LC-MS/MS; the modified lysine residues are underlined and in bold red as follows: K36, K75, K97, K117, K161, K186, K198, K214, K229, K249, K286, K300, K341, K375, K383, K499, K549, K584, K588 and K594.

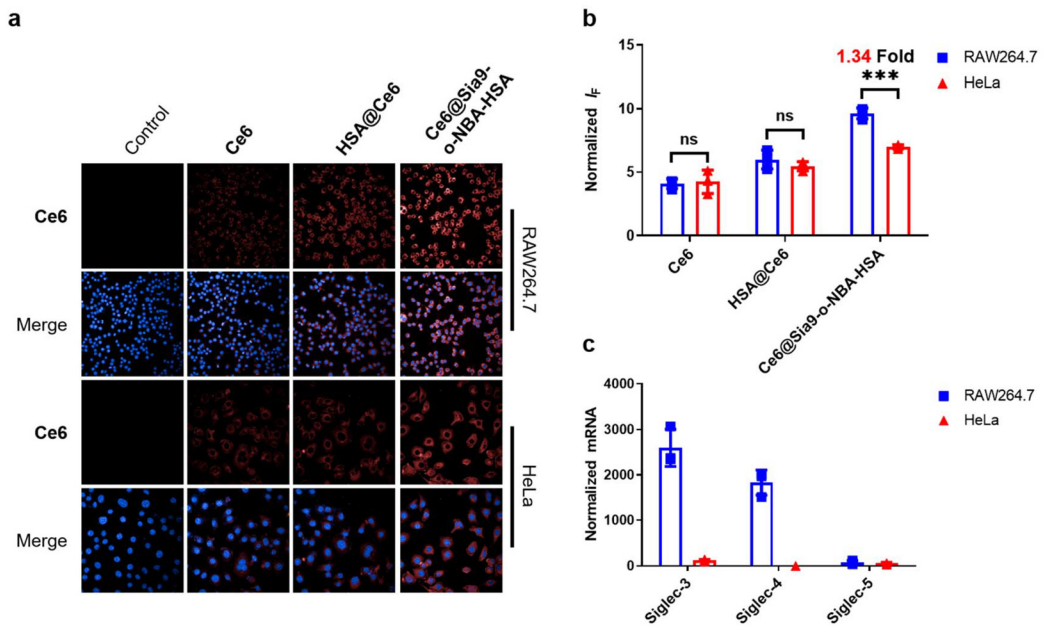

Fig. 3 (a) Fluorescence imaging and (b) quantification of different cells incubated with **Ce6**-encapsulated HSA derivatives (5 μM) for 1 h. (c) Relative mRNA expression level of three Siglec subtypes in RAW264.7 and HeLa determined by polymerase chain reaction. The excitation/emission channels used for **Ce6** and Hoechst 33342 (to stain nucleic acids) are 405/650–760 nm and 405/435–480 nm, respectively.

Conclusions

We synthesized two sialic acid derivatives bearing the ***o*-NBA** group at their 5- or 9-positions. The use of 365 nm light catalyzed the conjugation between both non-natural sugar derivatives with HSA. LC-MS/MS analysis indicated that the sugar-protein conjugates were successfully produced after light activation for 10 min, and a subsequent cell imaging assay was suggestive of different uptake efficiency by macrophages that highly express sialic acid-binding proteins. We anticipate that these derivatives will be useful for the light-mediated capture of sialic-acid binding proteins,[40,41] as well as for imaging applications in biological samples and live cells.[42–45] Relevant chemical biological studies are currently underway in our laboratory.

Data availability

All data associated with this study have been included in either the manuscript or the supplementary file associated with the manuscript.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the Natural National Science Foundation of China (NSFC) (No. 92253306, 82130099 and 22108077), the Shanghai Municipal Science and Technology Major Project (No. 2018SHZDZX03), the International Cooperation Program of Shanghai Science and Technology (No. 23490711600), the Fundamental Research Funds for the Central Universities (222201717003), the Programme of Introducing Talents of Discipline to Universities (B16017), the Open Funding Project of the State Key Laboratory of Bioreactor Engineering, State Key Laboratory of Drug Research (SKLDR-2023-KF-10) and Ministry of Education Key Laboratory on Signaling Regulation and Targeting Therapy of Liver Cancer (Naval Medical University) (Grant. 2023-MEKLLC-MS/ZD-00*) for financial support. TDJ wishes to thank the University of Bath and the Open Research Fund of the School of Chemistry and Chemical Engineering, Henan Normal University (2020ZD01) for support. The Research Center of Analysis and Test of East China University of Science and Technology is gratefully acknowledged for assistance in analytical experiments.

References

1 K. T. Schjoldager, Y. Narimatsu, H. J. Joshi and H. Clausen, *Nat. Rev. Mol. Cell Biol.*, 2020, **21**, 729–749.
2 C. Reily, T. J. Stewart, M. B. Renfrow and J. Novak, *Nat. Rev. Nephrol.*, 2019, **15**, 346–366.
3 A. Varki, *Glycobiology*, 2017, **27**, 3–49.
4 N. Berois, A. Pittini and E. Osinaga, *Cancers*, 2022, **14**, 645.
5 D. Thomas, A. K. Rathinavel and P. Radhakrishnan, *Biochim. Biophys. Acta, Rev. Cancer*, 2021, **1875**, 188464.
6 S. S. Pinho and C. A. Reis, *Nat. Rev. Cancer*, 2015, **15**, 540–555.
7 Y. Wang and H. Chen, *Oncogene*, 2023, **42**, 1970–1979.
8 A. A. Barkal, R. E. Brewer, M. Markovic, M. Kowarsky, S. A. Barkal, B. W. Zaro, V. Krishnan, J. Hatakeyama, O. Dorigo, L. J. Barkal and I. L. Weissman, *Nature*, 2019, **572**, 392–396.
9 C. Gao, L. Wisniewski, Y. Liu, B. Staal, I. Beddows, D. Plenker, M. Aldakkak, J. Hall, D. Barnett, M. K. Gouda, P. Allen, R. Drake, A. Zureikat, Y. Huang, D. Evans, A. Singhi, R. E. Brand, D. A. Tuveson, S. Tsai and B. B. Haab, *Clin. Cancer Res.*, 2021, **27**, 226–236.
10 R. B. Jones, A. D. Silva, K. E. Ankenbauer, C. M. Britain, A. Chakraborty, J. A. Brown, S. W. Ballinger and S. L. Bellis, *Glycobiology*, 2023, **33**, 626–636.
11 P. Schildhauer, P. Selke, M. S. Staege, A. Harder, C. Scheller, C. Strauss, R. Horstkorte, M. Scheer and S. Leisz, *Cells*, 2023, **12**, 2758.
12 P. Singh, A. Joon, M. Kumari, T. Singh, A. Bal, P. Maan and S. Ghosh, *Cell Biochem. Biophys.*, 2022, **80**, 781–793.
13 E. Scott, E. A. Goode, R. Garnham, K. Hodgson, M. Orozco-Moreno, H. Turner, K. Livermore, K. P. Nangkana, F. M. Frame, A. Bermudez, F. J. G. Marques, U. L. McClurg, L. Wilson, H. Thomas, A. Buskin, A. Hepburn, A. Duxfield, K. Bastian, H. Pye, H. M. Arredondo, G. Hysenaj, S. Heavey, U. Stopka-Farooqui, A. Haider, A. Freeman, S. Singh, E. W. Johnston, S. Punwani, B. Knight, P. McCullagh, J. McGrath, M. Crundwell, L. Harries, R. Heer, N. J. Maitland, H. Whitaker, S. Pitteri, D. A. Troyer, N. Wang, D. J. Elliott, R. R. Drake and J. Munkley, *J. Pathol.*, 2023, **261**, 71–84.
14 M. Zhou, S. Lv, Y. Hou, R. Zhang, W. Wang, Z. Yan, T. Li, W. Gan, Z. Zeng, F. Zhang and M. Yang, *Front. Immunol.*, 2022, **13**, 994874.
15 A. Gong, X. Zhao, Y. Pan, Y. Qi, S. Li, Y. Huang, Y. Guo, X. Qi, W. Zheng and L. Jia, *J. Cell Sci.*, 2020, **133**, jcs244020.
16 R. Hatanaka, E. Araki, M. Hane, S. Go, D. Wu, K. Kitajima and C. Sato, *Biochem. Biophys. Res. Commun.*, 2022, **608**, 52–58.
17 X. Zou, J. Lu, Y. Deng, Q. Liu, X. Yan, Y. Cui, X. Xiao, M. Fang, F. Yang, H. Sawaki, T. Sato, B. Tan, X. Lu, B. Feng, A. Kuno, H. Narimatsu, C. Gao and Y. Zhang, *Oncogene*, 2023, **42**, 516–529.
18 J. Scache, V. Rigolot, C. Lion, M. Mortuaire, T. Lefebvre, C. Biot and A. S. Vercoutter-Edouart, *Sci. Rep.*, 2022, **12**, 22129.
19 H. Wang, M. C. Sobral, D. K. Y. Zhang, A. N. Cartwright, A. W. Li, M. O. Dellacherie, C. M. Tringides, S. T. Koshy, K. W. Wucherpfennig and D. J. Mooney, *Nat. Mater.*, 2020, **19**, 1244–1252.

20 X. Fan, Q. Song, D. E. Sun, Y. Hao, J. Wang, C. Wang and X. Chen, *Nat. Chem. Biol.*, 2022, **18**, 625–633.
21 J. Han, R. Bhatta, Y. Liu, Y. Bo, A. Elosegui-Artola and H. Wang, *Nat. Commun.*, 2023, **14**, 5049.
22 Y. Zhong, L. Xu, C. Yang, L. Xu, G. Wang, Y. Guo, S. Cheng, X. Tian, C. Wang, R. Xie, X. Wang, L. Ding and H. Ju, *Nat. Commun.*, 2023, **14**, 7285.
23 H. Wang and D. J. Mooney, *Nat. Chem.*, 2020, **12**, 1102–1114.
24 W. Yi, P. Xiao, X. Liu, Z. Zhao, X. Sun, J. Wang, L. Zhou, G. Wang, H. Cao, D. Wang and Y. Li, *Signal Transduction Targeted Ther.*, 2022, **7**, 386.
25 H. Wu and J. Kohler, *Curr. Opin. Chem. Biol.*, 2019, **53**, 173–182.
26 K. Kozoriz, O. Shkel, K. T. Hong, D. H. Kim, Y. K. Kim and J.-S. Lee, *Acc. Chem. Res.*, 2023, **56**, 25–36.
27 A. D. Guo, K. N. Yan, H. Hu, L. Zhai, T. F. Hu, H. Su, Y. Chi, J. Zha, Y. Xu, D. Zhao, X. Lu, Y. J. Xu, J. Zhang, M. Tan and X. H. Chen, *Nat. Chem.*, 2023, **15**, 803–814.
28 A. D. Guo, D. Wei, H. J. Nie, H. Hu, C. Peng, S. T. Li, K. N. Yan, B. S. Zhou, L. Feng, C. Fang, M. Tan, R. Huang and X. H. Chen, *Nat. Commun.*, 2020, **11**, 5472.
29 A. D. Guo, K. H. Wu and X. H. Chen, *RSC Adv.*, 2021, **11**, 2235–2241.
30 H. Hu, W. Hu, A. D. Guo, L. Zhai, S. Ma, H. J. Nie, B. S. Zhou, T. Liu, X. Jia, X. Liu, X. Yao, M. Tan and X. H. Chen, *Nat. Commun.*, 2024, **15**, 1465.
31 W. Hu, Y. Yuan, C.-H. Wang, H.-T. Tian, A.-D. Guo, H.-J. Nie, H. Hu, M. Tan, Z. Tang and X.-H. Chen, *Chem*, 2019, **5**, 2955–2968.
32 H. Yang, L. Lu and X. Chen, *Biotechnol. Adv.*, 2021, **46**, 107678.
33 S. J. Moons, G. J. Adema, M. T. Derks, T. J. Boltje and C. Bull, *Glycobiology*, 2019, **29**, 433–445.
34 C. Bull, T. Heise, D. M. Beurskens, M. Riemersma, A. Ashikov, F. P. Rutjes, T. H. van Kuppevelt, D. J. Lefeber, M. H. den Brok, G. J. Adema and T. J. Boltje, *ACS Chem. Biol.*, 2015, **10**, 2353–2363.
35 Z. S. Chinoy, C. Bodineau, C. Favre, K. W. Moremen, R. V. Duran and F. Friscourt, *Angew. Chem., Int. Ed.*, 2019, **58**, 4281–4285.
36 X. Wang, S. Lang, Y. Tian, J. Zhang, X. Yan, Z. Fang, J. Weng, N. Lu, X. Wu, T. Li, H. Cao, Z. Li and X. Huang, *ACS Cent. Sci.*, 2020, **6**, 382–389.
37 X. Zheng, K. Sun, Y. Liu, X. Yin, H. Zhu, F. Yu and W. Zhao, *J. Controlled Release*, 2023, **353**, 675–684.
38 D. Hu, Z. Sheng, G. Gao, F. Siu, C. Liu, Q. Wan, P. Gong, H. Zheng, Y. Ma and L. Cai, *Biomaterials*, 2016, **93**, 10–19.
39 C. Reily, T. J. Stewart, M. B. Renfrow and J. Novak, *Nat. Rev. Nephrol.*, 2019, **15**, 346–366.
40 W.-T. Dou, Z.-Y. Qin, J. Li, D.-M. Zhou and X.-P. He, *Sci. Bull.*, 2019, **64**, 1902–1909.
41 W.-T. Dou, X. Wang, T. Liu, S. Zhao, J. Liu, Y. Yan, J. Li, C. Zhang, A. C. Sedgwick, H. Tian, J. L. Sessler, D. M. Zhou and X.-P. He, *Chem*, 2022, **8**, 1750–1761.
42 W.-T. Dou, P. Qiu, Y.-Y. Shi, L. Zhu, C. Guo, N. Li, Y. Zang, T.-T. Liu, S.-W. Zhao, Y.-F. Pan, L.-W. Dong, J. L. Sessler, Y.-X. Tan, J. Li, H.-Y. Wang, H. Tian and X.-P. He, *J. Am. Chem. Soc.*, 2023, **145**, 17377–17388.
43 W.-T. Dou, H.-H. Han, A. C. Sedgwick, G.-B. Zhu, Y. Zang, X.-R. Yang, J. Yoon, T. D. James, J. Li and X.-P. He, *Sci. Bull.*, 2022, **67**, 853–878.
44 X. Chai, H.-H. Han, A. C. Sedgwick, N. Li, Y. Zang, T. D. James, J. Zhang, X.-L. Hu, Y. Yu, Y. Li, Y. Wang, J. Li, X.-P. He and H. Tian, *J. Am. Chem. Soc.*, 2020, **142**, 18005–18013.
45 X.-P. He and H. Tian, *Chem*, 2018, **4**, 246–268.