ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2025, 61, 2277

Received 30th September 2024,
Accepted 2nd January 2025

DOI: 10.1039/d4cc05153j

rsc.li/chemcomm

The swelling-induced fractionation strategy to mediate cellulose availability and lignin structural integrity†

Dong Tian, *[a] Yu Zhang,[a] Tingjiao Wang,[a] Baiheng Jiang,[a] Miao Liu,[a] Li Zhao,[a] Jinguang Hu [b] and Fei Shen *[a]

We report a facile fractionation strategy using choline hydroxide (ChOH) based alkaline deep eutectic solvents (DES) for whole-component upgrading of bagasse. Through selective lignin and xylan dissolution, along with extensive biomass swelling, high-value lignin-carbohydrate complexes (LCC, with high β-O-4 bond content of 68.9/100 Ar) and high-purity xylan were extracted without compromising cellulose recovery and hydrolysis. This work provided the key to solving the technical contradiction between cellulose availability and lignin structural integrity.

Facing the challenges of petroleum resource scarcity and achieving carbon emission targets, seeking green alternative resources is imperative. Carbon-neutral lignocellulose biomass, mainly including cellulose, hemicellulose, and lignin, is an abundant biomass resource for the production of biofuels and biochemicals.[1] However, the entangled hydrogen bonding network and cross-linked chemical structure of biomass hinder the utilization of its major components.[2] Hence, developing efficient fractionation technology is crucial.

The present biomass refining process mainly consists of cellulose-first and lignin-first strategies. Traditional cellulose-first strategies focus on optimizing cellulose purity under harsh conditions, but it would cleave the precious aryl ether structure of lignin (Fig. 1a).[3,4] In contrast, the lignin-first strategy tended to directly depolymerize the natural structural lignin (high β-O-4 content) in biomass to high-value monophenol compounds.[5] However, the complicated catalytic system is prone to massive degradation of flexible carbohydrate structures (Fig. 1b). Among the product streams of these fractionation strategies, the emergence of the lignin-carbohydrate complex (LCC) is expected to reconcile this dilemma between fermentable sugar conversion and the aryl ether structure retention. Formed mainly from lignin and carbohydrates by covalent linking, LCC is a high-value amphiphilic polymer, which combines the advantages of both and is widely suitable for bioactive and biocompatible applications.[6] The mild extracting conditions of LCC were able to retain the structural advantages of both lignin and carbohydrates, but the great mass transfer resistance hindered yield. Therefore, seeking suitable solvents for LCC extraction and integrating them into the production process of highly hydrolyzed cellulose would be an attractive benefit.

Deep eutectic solvent (DES) can accomplish specific fractionation of biomass by disrupting hydrogen bonding networks and intermolecular interactions. A DES consists of a hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD).[7] In contrast to acidic DES, alkaline DES pretreatment can effectively retain the LCC structure and improve cellulose purity.[8,9] It was induced by cellulose swelling, covalent bond breakage, and the solvation reactions at elevated pH levels. Among the composition of alkaline DES, ChOH can be an excellent HBA

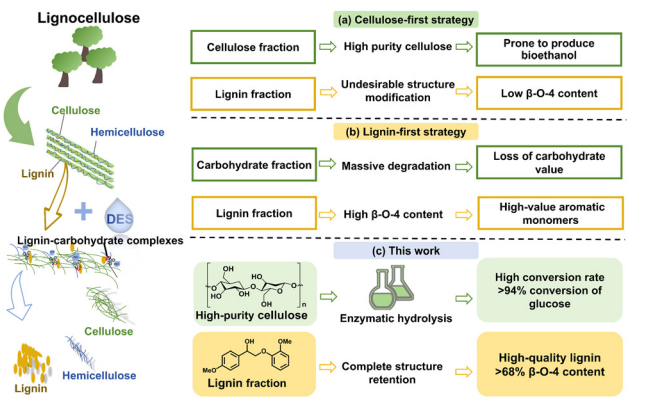

Fig. 1 (a) Cellulose-first strategy. (b) Lignin-first strategy. (c) The whole component utilization of biomass strategy proposed in this work using the tailored alkaline DES process.

[a] College of Environment Science, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China. E-mail: dongtian@sicau.edu.cn, fishen@sicau.edu.cn
[b] Department of Chemical and Petroleum Engineering, University of Calgary, 2500 University Dr. NW, Calgary, AB T2N 1N4, Canada

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4cc05153j

due to its high alkalinity and ionic properties. Ethylene glycol (Ely) or urea (Ur) can be used as favorable HBDs.[10,11] Alcohol-based HBDs can inhibit undesirable structural condensation and degradation during the reaction, which is favorable for the retention of lignin β-O-4 bonds.[12] Urea-based HBDs are selective for the solubilization of carbohydrates.[13] The employment of these two ChOH-based alkaline DESs is promising to achieve efficient co-production of LCC and carbohydrates.

This work proposed a ChOH-based alkaline DES fractionation process for the whole component utilization of bagasse (Fig. 1c). It was hypothesized that an alkaline DES could induce lignocellulose swelling, thereby selectively solubilizing LCC and xylan while enhancing the enzymatic accessibility of cellulose. Here, the effect of the constitution and concentration of alkaline DES on the LCC and xylan structure was systematically investigated. Also, the enzymatic hydrolysis potential of cellulose-rich solids was evaluated. The mass balance of the entire process route was finally tracked. In short, this alkaline DES process was designed to maximize the value of biomass refining.

The determination and analysis of the obtained biomass fractions were crucial in evaluating the effectiveness of the fractionation process. In Fig. 2a, compared to untreated bagasse (39.10%), the purity of cellulose solids reached 52.90% after pretreatment with 40% concentration of ChOH, while the purity of cellulose solids reached 57.8–76.7% after pretreatment with ChOH-based DES. The results suggested that an effective combination of alkaline DES could promote biomass fractionation. In addition, lignin and hemicellulose were solubilized by various concentrations of DES. Notably, pH and viscosity similarly increased with rising DES concentration (Fig. S1, ESI†), leading to an increase in cellulose purity and a decrease in solid yield. This meant that higher alkalinity DESs had stronger fractionation abilities.

The LCC and xylan composition analysis were further performed (Fig. 2b and c). Higher concentrations of DES resulted in high yields of LCC but were unfavorable to xylan yields. Moreover, with increasing DES concentrations, the obtained LCCs all showed a decrease in lignin content and an increase in xylan content. This suggested that high alkalinity DES enabled more xylan to be retained in the LCC fractions. Interestingly, the extracted LCC by Ch–Ely DES was dominated by lignin content, whereas the extracted LCC by Ch–Ur DES was dominated by xylan content, indicating that the alcohol-based HBD is more capable of retaining lignin, while urea HBD was better for xylan retention.[14] Therefore, the compositions and yields of the obtained LCCs and xylan could be modulated by varying the type of HBD and concentrations of the alkaline DESs.

The technical feasibility of these alkaline DESs on woody substrates of pine and oak were further compared. It was shown that LCC and xylan yield was much lower than that of the bagasse substrate (Fig. S2, ESI†). But higher DES concentration contributed xylan retention, which was in line with the above conclusion.

Characterization was conducted using XRD, WRV, and DP to comprehensively assess the enzymatic accessibility of the obtained cellulose fractions. All samples showed significant signals at 18° and 22.5° in the XRD patterns, indicating that the original cellulose crystal type was not altered (Fig. S3, ESI†). The CrI increased from 55.7% to 63.7–69.6% following the removal of amorphous lignin and hemicellulose. It showed that alkaline DES-induced cellulose swelling weakened the inter-crystal interaction and was more favorable for enzymatic digestion, which was more favorable for enzymatic hydrolysis. WRV was employed to evaluate the porosity within cellulose (Fig. S4, ESI†). Compared to untreated bagasse (228.5), the WRV of pretreated cellulose (327.2–372.9) showed significant increases, which could expose more enzymatic accessible active sites. DP was reduced to 782.8–978.0 for Ch–Ely and 808.7–1013.2 for Ch–Ur compared to the unmeasurable original bagasse. It was reported that the disruption of cellulose chains was able to weaken the fiber network and increase the number of reducing ends, which facilitated the subsequent enzymatic hydrolysis process.[15] In general, the obtained cellulose solids by alkaline Ch–Ely and Ch–Ur DESs possessed favorable potential for sugar platform conversion.

Enzymatic efficiency is the key indicator for evaluating the efficiency of the biomass fractionation technique.[16] As shown in Fig. 3, the original bagasse had only 18.71% glucose conversion and 10.11% xylose conversion *via* 96 h hydrolysis. After pretreatment with the alkaline Ch–Ely and Ch–Ur DES processes, the saccharification efficiencies of the obtained celluloses were both substantially increased. Sugar yields were also elevated with higher purity of cellulose solids, where 40% Ch–Ely and 40% Ch–Ur cellulose had the highest glucose yield and xylose yields,

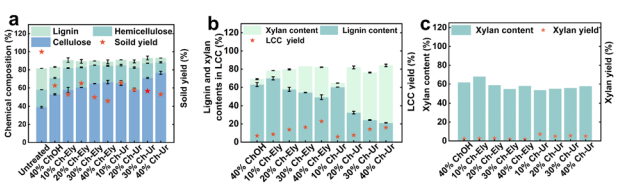

Fig. 2 (a) Chemical composition and solid recovery of bagasse. (b) Chemical compositions and yields of the LCC fraction. (c) Purity and yields of the xylan fraction.

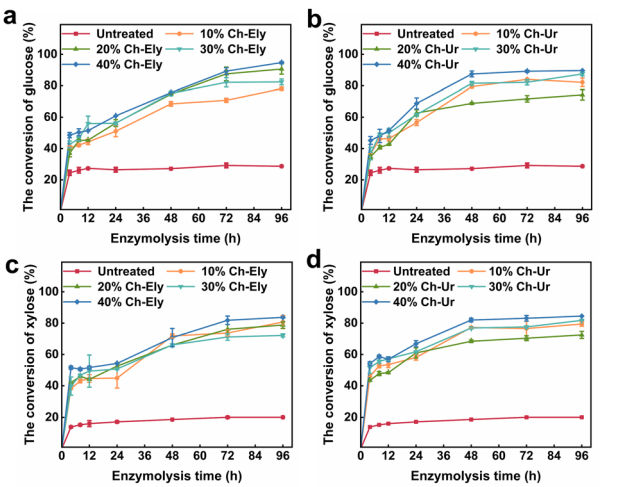

Fig. 3 The glucose conversion of cellulose and untreated bagasse (a) Ch–Ely and (b) Ch–Ur. The xylose conversion of cellulose and untreated bagasse. (c) Ch–Ely and (d) Ch–Ur.

reaching up to 94.72% and 84.52%. The results demonstrated the superiority of the swelling-induced fractionation strategy in obtaining easily digestible cellulose fractions.

To get more structural information on the obtained LCC and xylan, various characterization analyses were performed. ^{13}C NMR examined the carbon skeleton structure of LCC (Fig. S5, ESI†). In the 103–163 ppm range, both LCC and MWL showed obvious signals of the characteristic lignin units.[17] Moreover, the specific carbohydrate signals appeared in the 50–86 ppm range for LCC, proving that it retained both lignin and xylan complex structures.

Due to the overlap of lignin and carbohydrate partial signal peaks in ^{13}C NMR, 2D NMR was used to further elucidate the LCC structure (Fig. 4). In the side-chain region (δ_C/δ_H 50–90/2.5–6), Björkman LCC exhibited abundant β-D-xylopyranoside (X) and α-L-arabinofuranoside (Ara) signals, indicating that the carbohydrates in bagasse LCC were dominated by arabinoxylans, which was consistent with the structure of the extracted LCCs by an alkaline DES process.[18] Also, all the extracted LCCs showed the same signal peaks as MWL, mainly including methoxy (OMe), ether linkages (A) and phenyl coumarins (β-5), which also proved the structural integrity of lignin in LCCs. In particular, the retention of the β-O-4 ether bond is crucial to realize the whole component utilization of biomass. It was calculated that the β-O-4 bond contents of the four LCCs (51.4–68.9/100 Ar) were similar to that of the MWL (69.4/100 Ar). The content of β-O-4 in LCC extracted by the Ch–Ely was higher than the Ch–Ur, which also proved that the alcohol-based DES had a better ability to preserve the lignin structure. In addition, 40% DES tended to produce LCC with higher β-O-4 content than 10% DES, since higher alkalinity was more favorable for the retention of xylan in the LCC, thus reducing the lignin content.

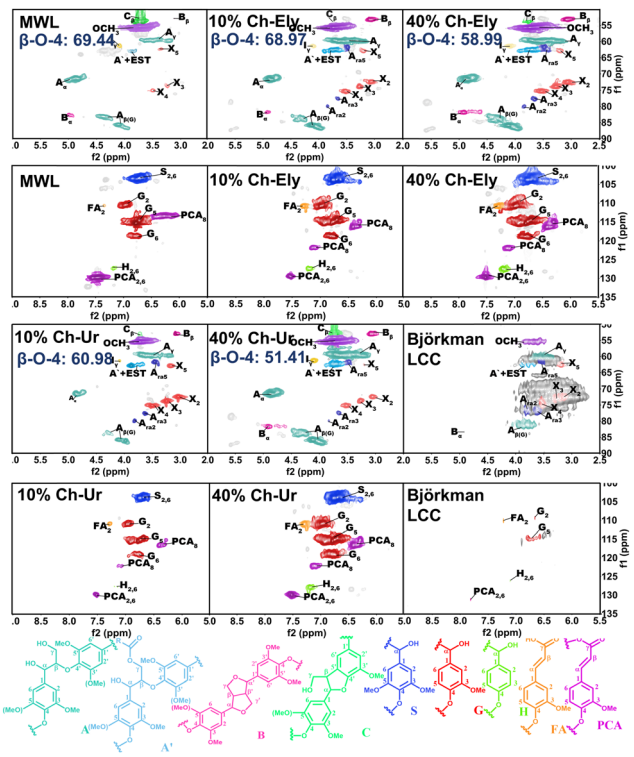

Fig. 4 Side-chain and aromatic regions in the 2D-HSQC NMR spectra of MWL and four different LCCs.

From the aromatic region (δ_C/δ_H 100–135/5.5–9), the obtained LCC exhibited the complete SGH basic structural unit of lignin. Meanwhile, as a typical herbaceous plant, abundant PCA and FA signals were detected in the spectra of bagasse LCC, indicating the covalent connection between lignin and hemicellulose in LCC through ether and ester bonds.[19,20] Representative PhGlc-linked for LCC signals were observed at δ_C/δ_H 105.0–90.0/5.3–4.0 ppm but further quantification of it seemed challenging due to its weak signal (Fig. S6, ESI†). In conclusion, the complete structure of the obtained LCC could be retained by the swelling-induced fractionation strategy.

The total phenolic hydroxyl (–OH) content of LCC was determined by the Folin–Ciocalteu method (Fig. S7, ESI†).[21] The –OH content of LCC decreased slightly due to the existence of xylan compared to MWL. The –OH content of LCC was also reduced with a lower concentration of DES, which was consistent with the changing pattern of lignin content of LCC. Overall, limited β-O-4 bond cleavage occurred thus retaining its natural reactivity. Thermogravimetric analysis was further performed (Fig. S8, ESI†). Two stages of weight loss were noted, which included the evaporation of residual water vapor in the LCC from 30–200 °C and the breakdown of carbohydrates and lignin aromatic compounds from 200–600 °C. 40% Ch–Ely and 40% Ch–Ur LCC exhibited lower carbon residue and faster rates of thermal degradation, which was attributed to the higher carbohydrate content. Therefore, the proposed tailored alkaline DES process could endow LCC with different structural properties by modulating their composition and concentration.

2D NMR of the extracted xylan is shown in Fig. 5. All the xylan spectra exhibited abundant carbohydrate signals, including X, Ara, and 4-*O*-methyl-α-D-glucuronide. This was consistent with the structure of herbaceous hemicellulose, in which xylan with arabinose and glucuronic acid moieties was the dominant side chain. Xylan resulting from 40% Ch–Ely or 10% Ch–Ur extraction showed stronger carbohydrate signal peaks, indicating that increasing the DES concentration or using urea HBD was more favorable for xylan extraction. Overall, the efficient extraction of LCC and xylan was realized by the proposed alkaline DES fractionation process.

Solvent recovery is vital for biorefineries. The spent DES waste liquid was rotary evaporated at 70 °C to recover the regenerated DES. Through FTIR and ^{1}H NMR characterization, it was shown that the neutral DES was obtained. It was demonstrated that the ChOH-based DES was converted to ChCl-based after neutralization with hydrochloric acid (Fig. S9, ESI†).

After 40% ChOH-Ur DES pretreatment, bagasse was fractionated into solid fraction and liquid fraction (Fig. 6). The solid fraction contained 35.24 g of cellulose, 2.83 g of lignin residues, and 8.09 g of xylan. The liquid fraction primarily consisted of soluble glucose, lignin, and hemicellulose. Additionally, 14.22 g LCC fraction and 2.54 g xylan with purities of 21.44% and 62.75% respectively were obtained by adjusting the properties

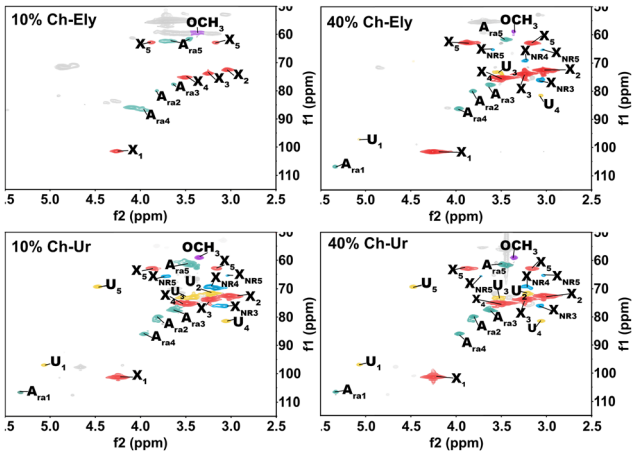

Fig. 5 2D-HSQC spectra of four different xylans.

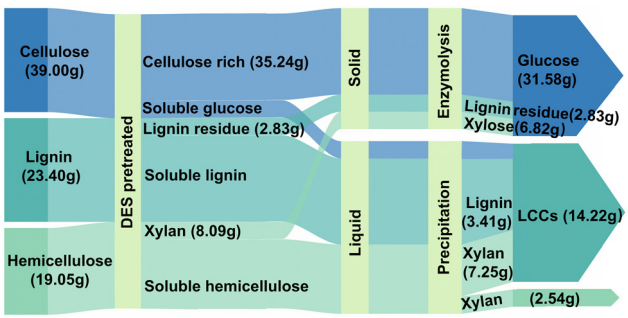

Fig. 6 Mass balance diagram of the alkaline DES fractionation process. Bagasse was cooked with 40% Ch–Ur at 130 °C for 3 h.

of the liquid fraction. The solid fraction (46.16 g) was hydrolyzed with cellulase and xylanase to produce 31.58 g of glucose, 6.82 g of xylose, and 2.83 g of lignin residue. Overall, through the proposed ChOH-based alkaline DES process, three value-added products were finally obtained.

Both the alkalinity and ionic nature of Ch–Ely and Ch–Ur DES contributed to the deconstruction of lignocellulose (Fig. S10, ESI†). The OH^- provided by ChOH was able to form new hydrogen bonds with the hydroxyl groups of the biomass fractions, resulting in the selective solubilization of amorphous lignin and hemicellulose. Ethylene glycol as a HBD could form α-etherified β-O-4 bonds (β′-O-4) through the capture of α-etherified carbon cation intermediates, hence acting to stabilize the lignin and effectively reducing condensation, which is more conducive to the retention of lignin fractions. The urea HBD was more alkaline, which was more conducive to hemicellulose solubilization under mild reaction conditions. Moreover, the alkaline DES could weaken the hydrogen bonding network between cellulose crystals to induce cellulose swelling, which exposed more enzyme active sites. In general, this tailored alkaline DES process achieves balanced regulation of cellulose accessibility and lignin structural integrity.

The tailored ChOH-based alkaline DES fractionation technique achieved the whole component utilization of bagasse biomass. The obtained cellulose exhibited high hydrolysis performance, yielding nearly 100% conversion at 5% solid loading. Meanwhile, the resulting LCC had high β-O-4 bond content, which is similar to that of MWL, while the obtained xylan possessed favorable carbohydrate structures. During the fractionation process, the composition and yield of the product could be modulated by varying the DES concentration and the type of HBD. This work provided three high-value products for downstream upgrading of carbohydrates and lignin, maximizing the application value of biomass.

This work was financially supported by the National Key R&D Program of China (2022YFE0207100), the National Natural Science Foundation of China (Grant No. 32472027 and 2478266) and the Department of Science and Technology of Sichuan Province (Grant No. 2022YFN0027 and 2023NSFSC0321).

Data availability

The data supporting this article have been included as part of the ESI,† including the original HPLC data for substrate chemical composition quantification.

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 L. Xu, M. Cao, J. Zhou, Y. Pang, Z. Li, D. Yang, S.-Y. Leu, H. Lou, X. Pan and X. Qiu, *Nat. Commun.*, 2024, **15**, 734.
2 E. Cooreman, T. Nicolaï, W. Arts, K. V. Aelst, T. Vangeel, S. V. den Bosch, J. V. Aelst, B. Lagrain, K. Thiele, J. Thevelein and B. F. Sels, *ACS Sustainable Chem. Eng.*, 2023, **11**, 5440–5450.
3 X. Shen and R. Sun, *Carbohydr. Polym.*, 2021, **261**, 117884.
4 W. Yang, Z. Mei, S. Feng, C. Li, J. Guo, H. Bian, H. Xiao, H. Dai, C. Hu and J. Han, *ACS Sustainable Chem. Eng.*, 2023, **11**, 10172–10182.
5 T. Renders, S. Van den Bosch, S. F. Koelewijn, W. Schutyser and B. F. Sels, *Energy Environ. Sci.*, 2017, **10**, 1551–1557.
6 B. Jiang, F. Shen, Y. Jiang, M. Huang, L. Zhao, Y. Lei, J. Hu, D. Tian and F. Shen, *Carbohydr. Polym.*, 2024, **323**, 121452.
7 Y. Ma, Y. Yang, T. Li, S. Hussain and M. Zhu, *Green Chem.*, 2024, **26**, 3627–3669.
8 H. Yu, Y. Wang, C. Yan, D. Yu, L. Chen, Q. Hu, Y. Yao, T. Mu and Z. Xue, *ACS Sustainable Resour. Manage.*, 2024, **1**, 1931–1942.
9 C. He, X. Li, F. Luo, C. Mi, A. Zhan, R. Ou, J. Fan, J. H. Clark and Q. Yu, *ACS Sustainable Chem. Eng.*, 2024, **12**, 11327–11337.
10 Y. Zhong, J. Wu, H. Kang and R. Liu, *Green Chem.*, 2022, **24**, 2464–2475.
11 Y. Jiang, F. Shen, B. Jiang, Y. Hu, J. Hu, M. Huang, L. Zhao, H. Wu, D. Tian and F. Shen, *Chem. Eng. J.*, 2024, **479**, 147610.
12 J. Cheng, X. Zhou, C. Huang, C. G. Yoo, X. Meng, G. Fang, A. J. Ragauskas and C. Huang, *Green Chem.*, 2024, **26**, 8298–8314.
13 S. P. Simeonov and C. A. M. Afonso, *RSC Adv.*, 2016, **6**, 5485–5490.
14 Y. Liu, N. Deak, Z. Wang, H. Yu, L. Hameleers, E. Jurak, P. J. Deuss and K. Barta, *Nat. Commun.*, 2021, **12**, 5424.
15 X.-J. Shen, J.-L. Wen, Q.-Q. Mei, X. Chen, D. Sun, T.-Q. Yuan and R.-C. Sun, *Green Chem.*, 2019, **21**, 275–283.
16 M. Hassanpour, M. Abbasabadi, L. Gebbie, V. S. J. Te'o, I. M. O'Hara and Z. Zhang, *ACS Sustainable Chem. Eng.*, 2020, **8**, 10380–10388.
17 H. Zhang, H. Zhao, Y. Yang, H. Ren and H. Zhai, *Green Chem.*, 2022, **24**, 2212–2221.
18 X. Wang, J. Pu, Y. Liu, C. Qin, S. Yao, S. Wang and C. Liang, *J. Agric. Food Chem.*, 2024, **72**, 10206–10217.
19 D. Sun, Z.-W. Lv, J. Rao, R. Tian, S.-N. Sun and F. Peng, *Carbohydr. Polym.*, 2022, **281**, 119050.
20 A. Kirui, W. Zhao, F. Deligey, H. Yang, X. Kang, F. Mentink-Vigier and T. Wang, *Nat. Commun.*, 2022, **13**, 538.
21 T. N. Nikolaeva, P. V. Lapshin and N. V. Zagoskina, *Russ. J. Bioorg. Chem.*, 2022, **48**, 1519–1525.

