Bioconjugate Chemistry

Technical Note

pubs.acs.org/bc

Microwave Heating for the Rapid Generation of Glycosylhydrazides

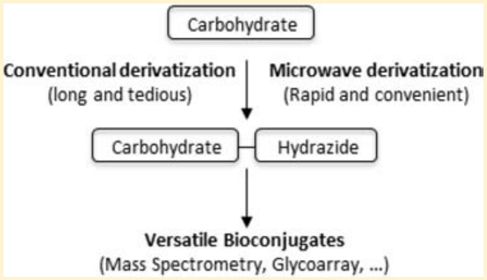
F. Mallevre,[†] A. Roget,*[,†] T. Minon,[†] Y. Kervella,[‡] D. Ropartz,[§] M. C. Ralet,[§] H. Canut,[∥,⊥] and T. Livache[†]

[†]CREAB and [‡]LEMOH, INAC CEA Grenoble, UMR SPrAM 5819 (CEA, CNRS, UJF), 17 rue des Martyrs, 38054 Grenoble Cedex 9, France

[§]INRA, UR 1268 Biopolymères Interactions Assemblages, Rue de la Géraudière, B.P. 71627, 44316 Nantes Cedex 3, France

[∥]Université de Toulouse, UPS, Laboratoire de Recherche en Sciences Végétales, and [⊥]CNRS, UMR 5546, B.P. 42617 Auzeville, 31326 Castanet-Tolosan, France

ABSTRACT: Conditions for simple derivatization of reducing carbohydrates via adipic acid dihydrazide microwave-assisted condensation are described. We demonstrate with a diverse set of oligo- and polysaccharides how to improve a restrictive and labor intensive conventional conjugation protocol by using microwave-assisted chemistry. We show that 5 min of microwave heating in basic or acidic conditions are adequate to generate, in increased yields, intact and functional glycosylhydrazides, whereas hours to days and acidic conditions are generally required under conventional methods.



INTRODUCTION

Carbohydrates are complex biomolecules, found in cell walls and on cell membranes, which have emerged in the past decade as critical actors in many biological mechanisms, such as cell signaling and differentiation, immune and tumoral responses, and microbial infections.[1,2] Many analytical techniques are consequently implemented in glycomics for elucidating the versatile roles of carbohydrates, such as mass spectrometry, chromatography, glycoarray, electromigration, enzyme-linked immunosorbent assay, or phage display.[3,4] Therefore, the generation of glycoconjugates for purification, labeling, or immobilization is crucial. Unfortunately, current derivatization methods at the carbohydrate reducing end are generally long and tedious, so much that a large number of techniques have been described with a few, however, being regarded as standard nowadays.[5] Main approaches are based on Staudinger ligation,[6] reductive amination,[7−10] click chemistry,[11,12] Kochetkov reaction,[13] or carbonyl condensation.[14−16] All have to overcome the carbohydrate structural and physicochemical heterogeneity as well as the thermodynamic disadvantage of the reactive open-chain forms; common sources of complicated processes, poor coupling yields, or long reaction times.[5−16] Although challenging, we think that the addition of linkers via conjugation of the reducing terminal is the best way of generating versatile glycoconjugates. In line with these works and encouraged by the large choice of commercial ester-activated molecules (e.g., NHS-thiol, NHS-pyrrole, NHS-biotin, NHS-nanoparticle, NHS-oligonucleotide), we are convinced that rapid and facile generation of glycosylhydrazides is useful for glycomics.

Microwave technology is an increasingly popular alternative for chemistry in the biosciences.[17−19] It is broadly described as an efficient tool to accelerate chemical reactions and to reduce byproduct formation. The primary application domains are organic synthesis,[20] green chemistry,[21] peptide synthesis,[22] and hydrolysis or conversion of carbohydrates.[23,24] However, the efficiency of microwave heating is still rarely applied in glycomics to the generation of glycoconjugates. Brun and co-workers, as well as Bejugam and Flitsch, produced various derivatives from glycosylamines generated by conversion under microwave-enhanced Kochetkov amination.[25,26] Yates and co-workers, as well as Zhou and co-workers, immobilized sugars to amino-derivatized surface via microwave-enhanced reductive amination.[27,28] Bouillon and co-workers produced glycosyloligonucleotides via microwave-enhanced click chemistry from glycosyl azide derivatives.[29] As far as we are aware, no direct derivatization with reducing carbohydrates has been realized under microwave conditions. Considering the numerous functionalized carbohydrate based applications and the promises of the microwave technology, we explored the generation of glycosylhydrazides via microwave-enhanced carbonyl condensation with an adipic acid dihydrazide bifunctional linker.

In this report, we propose the use of microwave heating for the enhancement and expansion of the hydrazone based conjugation method with various reducing oligo- and polysaccharides. We propose a rapid, efficient, and convenient solution for the generation of intact, functional, and versatile glycosylhydrazides characterized by mass spectrometry and surface plasmon resonance based glycoarray.

MATERIALS AND METHODS

Carbohydrate Derivatization. Galactan, arabinan, and galacturonan linear oligosaccharides (degree of polymerization

Received: December 14, 2012
Revised: May 24, 2013
Published: June 4, 2013

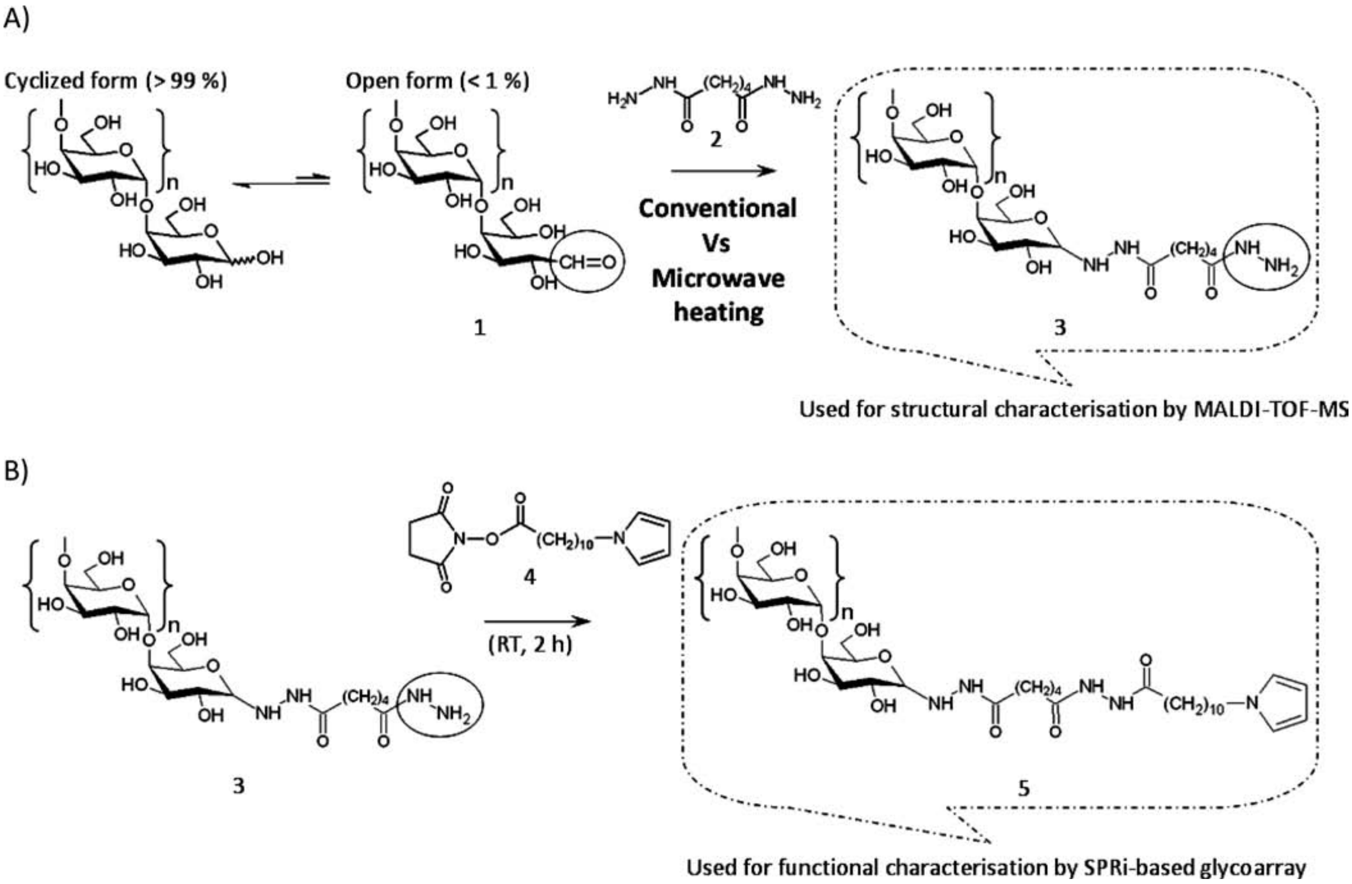


Figure 1. Scheme of the strategy as presented in this paper. Galactan oligosaccharides were conjugated at their reducing-end by condensation of an adipic acid dihydrazide linker under either conventional or microwave conditions. Generated glycosylhydrazides were characterized by mass spectrometry, and further coupled with NHS-pyrrole, then characterized by surface plasmon resonance after immobilization on gold surface. (A) Scheme of investigated conjugation reaction. (B) Scheme of hydrazide/ester-activated reaction. (1: open galactan, 2: adipic acid dihydrazide linker, 3: galactan hydrazide, 4: NHS-pyrrole grafting agent, 5: pyrrolylated-galactan).

4–12, centered on 6–7) produced and purified as previously described by Ralet et al.,[30] as well as heparin and chondroitin sulfate glycosaminoglycans (Sigma), were modified at their reducing-end by a two-step approach as previously explored by Bartoli et al. and Mercey et al.[15,31] As shown in Figure 1, carbohydrates were first conjugated to a dihydrazide linker by condensation under either conventional or microwave heating conditions. Glycosylhydrazides were then directly used for mass spectrometry analysis or further coupled with NHS-pyrrole for glycoarray assays. Briefly, carbohydrates were dissolved at 1 mM in sodium acetate buffer (100 mM, pH 5 or 6) or in borate buffer (100 mM, pH 8 or 9) with 50 mM of adipic acid dihydrazide (ADH, Sigma) and incubated at 56 °C during hours to days (from 6 to 72 h), as previously described for conventional derivatization. In the alternative approach based on microwave heating, carbohydrates at 1 mM in sodium acetate buffer (100 mM, pH 5 or 6) or in borate buffer (100 mM, pH 8 or 9) were incubated with 50 mM of ADH in an open-vessel Discover CEM microwave reactor at 56 °C, in SPS mode at 50 W with continuous cooling and medium stirring during some minutes (from 2 to 30 min). Glycosylhydrazides were then dissolved at 5 mM in PBS and incubated 2 h at room temperature with NHS-pyrrole (synthesized in the laboratory) at 10 mM in DMSO, in a final PBS/DMSO solution in a 1:1 ratio (v/v). Generated products (glycosylhydrazides and pyrrolylated-glycoconjugates) were purified on PD MidiTrap G-10 columns (GE Healthcare), freeze–dried, and stored at −20 °C before use.

Mass Spectrometry Analyses. Glycosylhydrazides from conventional or microwave conjugation were structurally characterized by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. MALDI-TOF-MS experiments were performed using an Autoflex III TOF/TOF mass spectrometer (Bruker Daltonics), equipped with a Smartbeam laser (355 nm, 200 Hz) set to positive ionization mode with reflector detection. The m/z range was set to 600–2200. DHB/DMA matrix was prepared as previously described by Ropartz et al.[32] 1 μL of each carbohydrate sample (at 500 μg/mL) was deposited on a polished steel MALDI target plate and mixed with the matrix solution in a 1:1 ratio (v/v). Acquisition parameters (laser power, pulsed ion extraction, etc.) were optimized for each sample. Mass data were recorded using FlexControl and processed using FlexAnalysis (Bruker Daltonics).

Glycoarray Generation. Pyrrolylated-glycoconjugates were immobilized on prisms bearing a gold surface ($n = 1.717$ at $\lambda = 633$ nm, GenOptics, Horiba Scientific) by electrochemical directed polymerization as previously described.[15,31] Glycoconjugates were solubilized in sodium phosphate buffer (0.1 M, pH 6.8, 10% glycerol) containing free pyrrole (Tokyo Kasei) at 20 mM. A polypyrrole film was then formed on the gold surface by electro-copolymerization, using a difference of potential of 2 V applied for 0.2 s between a working electrode (gold layer)

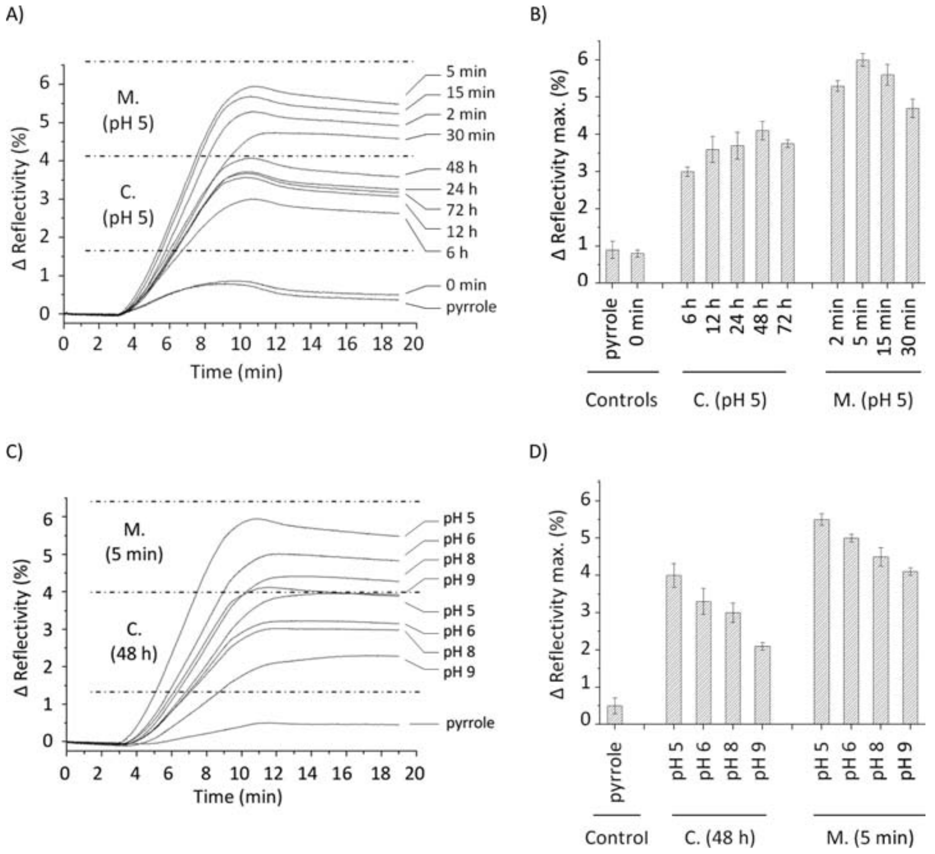


Figure 2. Comparative surface plasmon resonance results for galactans. Galactan hydrazides generated by conventional (C) or microwave (M) chemistries under various conditions of time (from minutes to days) and pH (acidic and basic) were characterized using SPRi via specific LM5–galactan interaction assays. (A) Sensorgram of coupling time course. (B) Comparative histogram of coupling time course. (C) Sensorgram of coupling pH course. (D) Comparative histogram of coupling pH course. Error bars represent the calculated standard deviation (SD).

and a counter electrode (platinum modified pipet tip). Tip, generating a spot of about 300 μm in diameter, was emptied and rinsed with water after each electro-polymerization. At the end of the glycoarray fabrication process, the prism was rinsed with water, dried, and stored at 4 °C.

Surface Plasmon Resonance Imaging Analyses. The functionality of generated glycoconjugates was characterized by interaction assays via surface plasmon resonance imaging experiments. SPRi analyses were performed using a SPRi-PlexII apparatus (GenOptics, Horiba Scientific, $\lambda = 650$ nm) in a Kretschmann optical configuration as previously described by Fiche et al.[33] Biomolecular interactions were carried out in a 10 μL hexagonal flow cell in running buffer (HEPES 10 mM, NaCl 150 mM, Tween-20 0.005%, pH 7.4) at 70 μL/min and 25 °C. Prior to experiment, nonspecific interactions on the biochip surface were blocked by injecting 1% bovine serum albumin (BSA, Sigma). For galactan, arabinan, and galacturonan oligosaccharides, specific antibodies LM5, LM6, and LM19 (Plantprobes) were used for interaction assessments, respectively. For heparin and chondroitin sulfate glycosaminoglycans, interferon-γ and antichondroitin sulfate antibody (Sigma) were used. Regeneration of the biochip surface was performed using a denaturing 2 M guanidine hydrochloride (Sigma) fresh solution. All buffers were filtered through a 0.22 μm filter and degassed prior to use. SPRi data were recorded using SPRiview software (GenOptics, Horiba Scientific) and processed using Origin software (OriginLab).

RESULTS AND DISCUSSION

In this technical note, our efforts have focused on the improvement and expansion of a carbonyl condensation based method for the generation of versatile glycosylhydrazides. As current protocols for the derivatization of reducing carbohydrates are long and labor intensive, we sought to develop a new one based on microwave-assisted chemistry. To this end, galactan oligosaccharides ([→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→]) were modified following the two-step process described in Figure 1. Our strategy includes the addition of ADH under either conventional or microwave various time and pH conditions, and the coupling with NHS-pyrrole for immobilization, then SPRi-based glycoarray assays with the specific LM5 antibody.

For the reaction time study, galactans were modified at pH 5 from 2 to 30 min under microwave heating (56 °C, 50 W) or from 6 to 72 h under conventional heating (56 °C). As shown in Figure 2 Sensorgram A, strong and specific galactan–LM5 interactions with characteristic high association and low disassociation steps are observed by SPRi, attesting to the success of the conjugation as well as the functionality of the generated glycoconjugates. In addition, SPRi signals are repeatedly higher with microwave-prepared galactans, indicating a clear, positive effect of microwave heating on the conjugation reaction. This gain may be explained by an increase in the number of carbohydrates per spot. Indeed, due to the theoretical enhancement of the mutarotation phenomenon under microwave heating,[23,34] an increase in the amount of

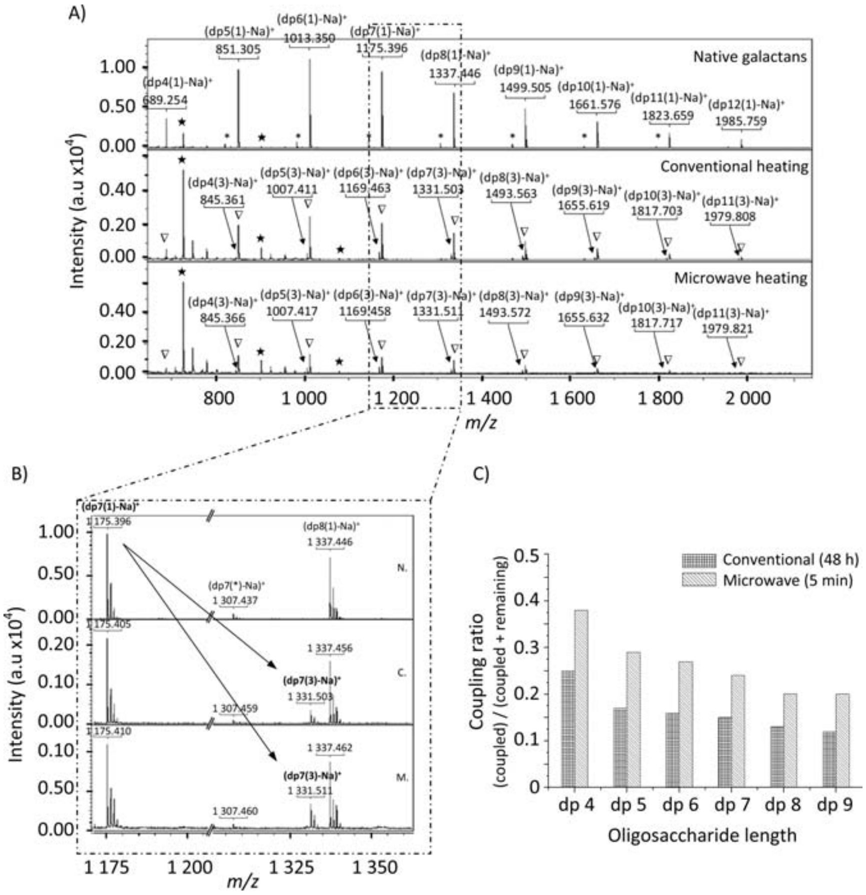


Figure 3. Comparative mass spectrometry results for galactans. Native galactans as well as galactan hydrazides generated by conventional (48 h at pH 5) or microwave (5 min at pH 5) chemistries were characterized using MALDI-TOF-MS in the positive ion mode. (A) Spectra of native galactans and galactan hydrazides (m/z 600−2200). (B) Zoom (m/z 1170−1360). On spectra, products are named as a function of their length (dp from 4 to 12), type (1 for native and 3 for coupled, in accordance with Figure 1), and ion (Na^+). Matrix clusters are marked by★, arabinose contaminated galactans by *, and remaining galactans by ▽. (C) Comparative histogram of coupling ratios determined as (coupled)/(coupled + remaining) proportions after intra profile peak integration.

reactive open forms leading to more glycosylhydrazides generated, then higher SPRi results, may be supposed. As shown in Figure 2 Histogram B, gathering the maximum of reflectivity variations (ΔR_{max}) obtained by SPRi for each coupling condition, long periods as 48 h (with ΔR_{max} = 4.1%) were found to be the most advantageous for conventional heating. Comparatively, very short periods such as 5 min (with ΔR_{max} = 6%) were optimal under microwave conditions. Taken together, these results demonstrate the possibility to rapidly generate functional galactan hydrazides in only 5 min under microwave heating with, moreover, a conjugation efficiency enhanced by about 30%.

For the pH condition study, galactans were modified at two acidic (5, 6) and two basic (8, 9) pH values during 5 min under microwave heating (56 °C, 50 W) or 48 h under conventional heating (56 °C). As shown in Figure 2 Sensorgram C, strong and specific galactan−LM5 interactions are observed with again higher signals for microwave-prepared glycoconjugates. Unsurprisingly the optimal pH for the conjugation of galactan oligosaccharides is acidic. As shown in Figure 2 Histogram D, a significant SPRi signal decrease is observed when the pH is increased. ΔR_{max} values diminish from 4% at pH 5 to 2.1% at pH 9 for the conventional conjugation, and from 5.5% to 4% for the microwave one. The pH influence is diminished with microwave-assisted chemistry. Conjugation reactions in basic conditions are then conceivable and could be particularly helpful for the derivatization of pH sensitive carbohydrates or to avoid the known hydrazide based glycoconjugate hydrolysis under acidic conditions.[34] Taken together, these results demonstrate that pH 5 is optimal for the generation of functional galactan hydrazides but efficient conjugations are completely possible in basic conditions, especially under microwave heating.

We have demonstrated the possibility to generate rapidly functional galactan hydrazides via microwave heating during 5 min at pH 5. To further characterize the glycoconjugates generated in these conditions, native galactans, as well as conventional and microwave galactan hydrazides, were analyzed by MALDI-TOF-MS. As shown in Figure 3 Profile A, the distribution of native galactans ranges from dp 4 to dp 12, centered on dp 6−7. As previously described in the literature, extracted galactans exhibit a minor part of arabinans,[30,32] characterized by a m/z shift of +132 due to the presence of one additional arabinose monosaccharide (Figure 3 Profile A and Zoom B). In all cases, for conventional as well as microwave methods, generated glycoconjugates are clearly identified with a m/z shift of +156 from the native references corresponding to the ADH addition. With consecutive galactans varying by a m/z shift of +162, the peak of dp 7 galactan hydrazides is thus annotated close to the dp 8 native galactans one, for example

(Figure 3 Zoom B). In addition, no byproduct and abnormal fragment are observed. In combination, these results attest to the structural integrity of the generated glycoconjugates by the two methods. Coupling ratios, defined as (coupled)/(coupled + remaining) proportions, were then determined for all species after intraprofile peak integration. As shown in Figure 3 Histogram C, higher yields are associated with shorter oligosaccharides. However, coupling ratios under microwave conditions are systematically 30−40% higher than the conventional ones, testifying to the enhancement of the conjugation reaction under microwave heating. In direct correlation with the previous SPRi signal gains observed, these results are reasonably explained by a mutarotation rate increase and a higher availability of the reactive carbohydrate open-chain forms during the microwave-assisted reaction of conjugation. Taken together, MALDI-TOF-MS results demonstrate the structural integrity of the generated glycoconjugates as well as the enhancement of the coupling ratios under microwave conditions.

Considering that carbohydrates have an important structure/property relationship, various oligo- and polysaccharides were conjugated, then used for SPRi based-glycoarray assays. In the range of cell wall pectic polymers, arabinans ([→5)-*α*-L-Ara*f*-(1→5)-*α*-L-Ara*f*-(1→]) and galacturonans ([→4)-*α*-D-GalA*p*-(1→4)-*α*-D-GalA*p*-(1→]) were used in addition to galactans. In the range of glycosaminoglycans, heparin ([→4)-*α*-*HexAp*-(1→4)-*α*-GlcNAc*p*-(1→]) and chondroitin sulfate ([→4)-*β*-*HexAp*(1→4)-*β*-GalNAc*p*-(1→]) were also tested. As shown in Table 1, the benefits observed under microwave derivatization conditions are consistent with these other candidates. Thus, results demonstrate versatility and reliability of the developed microwave method for the conjugation of various short (700−2000 Da) or long (15−60 kDa), neutral or charged, pyranosyl- or furanosyl-containing, and plant or mammalian carbohydrates.

Table 1. Comparative Surface Plasmon Resonance Results for Various Carbohydrates[a]

	chemistry interaction	conventional 48 h	microwave 5 min
Δ Reflectivity maximum (%) (SD: ± 0.2)	LM5−Galactan	4.1	6
	LM6−Arabinan	0.9	1.2
	LM19−Galacturonan	4.5	5.5
	Interferon-γ−Heparin	6	8
	IgM anti CS−CS	1.5	2.5

[a] Plant oligosaccharides (galactan, arabinan, and galacturonan) and mammalian glycosaminoglycans (heparin and chondroitin sulfate) were functionalised under optimal conventional or microwave conditions then used for SPRi-based glycoarray assays. Results of the specific biomolecular interactions monitored are presented.

CONCLUSION

We report the use of microwave heating for the improvement and expansion of a conventional hydrazone based carbohydrate conjugation method. Mass spectrometry and surface plasmon resonance analyses have been proposed to structurally and functionally characterize the effect of microwave irradiations on generated glycoconjugates. We have established that optimal conditions for the generation of fully functional and intact glycosylhydrazides are 5 min at pH 5 under microwave heating, whereas hours to days are generally required under conventional methods. Moreover, we observed a significant enhancement in the coupling ratios by about 30−40% under microwave conditions. We also validate these results with various oligosaccharides and glycosaminoglycans, attesting to the reliability and versatility of the developed microwave approach. In addition, we report the possibility to efficiently generate glycoconjugates under basic conditions, facilitating the way to functionalize pH sensitive carbohydrates. Thus, the microwave-assisted chemistry proposed herein for the generation of glycosylhydrazides outperforms the conventional one in terms of rapidity, efficiency, and versatility. We are moreover convinced that it may be easily and successfully applied to a large range of other bioconjugation methodologies involving derivatization issues.

AUTHOR INFORMATION

Corresponding Author

*E-mail: andre.roget@cea.fr; Tel.: +33 4 38 78 66 78; Fax: +33 4 38 78 51 45.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the ANR and the Genomic & Plant Biotechnologies Program for the financial support (ANR-GENOM-BTV-010-01). We thank Genoplante-Valor for the consortium agreement. We also thank Yanxia Hou-Broutin and Hillary Wehry for critical reading of the manuscript.

REFERENCES

(1) Varki, A., and Lowe, J. B. (2009) Biological roles of glycans. *Essentials of glycobiology*. (Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. E., Eds.) pp 80−81, Chapter 6, Cold Spring Harbor Laboratory Press, New York.

(2) Van Kooyk, Y., and Rabinovich, G. A. (2008) Protein-glycan interactions in the control of innate and adaptative immune responses. *Nat. Immunol. 9*, 593−601.

(3) Rakus, J. F., and Mahal, L. K. (2011) New technologies for glycomic analysis: toward a systematic understanding of the glycome. *Annu. Rev. Anal. Chem. 4*, 367−392.

(4) Park, S., Lee, M. R., and Shin, I. (2008) Chemical tools for functional studies of glycans. *Chem. Soc. Rev. 37*, 1579−1591.

(5) Harvey, D. J. (2011) Derivatization of carbohydrates for analysis by chromatography; electrophoresis and mass spectroscopy. *J. Chromatogr., B 879*, 1196−1225.

(6) Loka, R. S., Sadek, C. M., Romaniuk, N. A., and Cairo, C. W. (2010) Conjugation of synthetic N-acetyl-lactosamine to azide-containing proteins using the Staudinger Ligation. *Bioconjugate Chem. 21*, 1842−1849.

(7) Bohorov, O., Andersson-Sand, H., Hoffmann, J., and Blixt, O. (2006) Arraying glycomics: a novel bi-functional spacer for one-step microscale derivatization of free reducing glycans. *Glycobiology 16* (12), 21−27.

(8) Unterieser, I., and Mischnick, P. (2011) Labeling of oligosaccharides for quantitative mass spectrometry. *Carbohydr. Res. 346*, 68−75.

(9) Xia, B., Kawar, Z. S., Ju, T., Alvarez, R. A., Sachdev, G. P., and Cummings, R. D. (2005) Versatile fluorescent derivatization of glycans for glycomic analysis. *Nat. Methods 2* (11), 845−850.

(10) Bårström, M., Bengtsson, M., Blixt, O., and Norberg, T. (2000) New derivatives of reducing oligosaccharides and their use in

enzymatic reactions: efficient synthesis of sialyl Lewis a and sialyl dimeric Lewis x glycoconjugates. *Carbohydr. Res. 328*, 525−531.

(11) Tanaka, T., Nagai, H., Noguchi, M., Kobayashi, A., and Shoda, S. I. (2009) One-step conversion of unprotected sugars to β-glycosyl azides using 2-chloroimidazolinium salt in aqueous solution. *Chem. Commun.*, 3378−3379.

(12) Halila, S., Manguian, M., Fort, S., Cottaz, S., Hamaide, T., Fleury, E., and Driguez, H. (2008) Syntheses of well-defined glyco-polyorganosiloxanes by "click" chemistry and their surfactant properties. *Macromol. Chem. Phys. 209*, 1282−1290.

(13) Shin, I., Jung, H. J., and Lee, M. R. (2001) Chemoselective ligation of maleimidosugars to peptides/protein for the preparation of neoglycopeptides/neoglycoprotein. *Tetrahedron Lett. 42*, 1325−1328.

(14) Bartoli, J., Roget, A., and Livache, T. (2012) Polypyrrole-oligosaccharide microarray for the measurement of biomolecular interactions by surface plasmon resonance imaging. *Carbohydrate Microarrays: Methods and Protocols. Methods in Molecular Biology 808 (Chevolot Ed.)* pp 69−86, Chapter 5, Humana Press, New York.

(15) Ridley, B. L., Spiro, M. D., Glushka, J., Albersheim, P., Darvill, A., and Mohnen, D. (1997) A method for biotin labelling of biologically active oligogalacturonides using a chemically stable hydrazide linkage. *Anal. Biochem. 249*, 10−19.

(16) Flinn, N. S., Quibell, M., Monk, T. P., Ramjee, M. K., and Urch, C. J. (2005) A single-step method for the production of sugar hydrazides: intermediates for the chemoselective preparation of glycoconjugates. *Bioconjugate Chem. 16*, 722−728.

(17) Kappe, C. O., and Dallinger, D. (2009) Controlled microwave heating in modern organic synthesis: highlights from the 2004−2008 literature. *Mol. Divers. 13*, 71−193.

(18) Collins, J. M., and Leadbeater, N. E. (2007) Microwave energy: a versatile tool for the biosciences. *Org. Biomol. Chem. 5*, 1141−1150.

(19) Strauss, C. R., and Rooney, D. W. (2010) Accounting for clean, fast and high yielding reactions under microwave conditions. *Green Chem. 12*, 1340−1344.

(20) Dallinger, D., and Kappe, C. O. (2007) Microwave-assisted synthesis in water as solvent. *Chem. Rev. 107*, 2563−2591.

(21) Bassyouni, F. A., Abu-Bakr, S. M., and Rehim, M. A. (2012) Evolution of microwave irradiation and its application in green chemistry and biosciences. *Res. Chem. Intermed. 38*, 283−322.

(22) Pedersen, S. L., Tofteng, A. P., Malik, L., and Jensen, K. J. (2012) Microwave heating in solid-phase peptide synthesis. *Chem. Soc. Rev. 41*, 1826−1844.

(23) Corsaro, A., Chiacchio, U., Pistara, V., and Romeo, G. (2004) Microwave-assisted chemistry of carbohydrates. *Curr. Org. Chem. 8*, 511−538.

(24) Richel, A., Laurent, P., Wathelet, B., Wathelet, J. P., and Paquot, P. (2011) Microwave-assisted conversion of carbohydrates. State of the art and outlook. *C. R. Chim. 14*, 224−234.

(25) Brun, M. A., Disney, M. D., and Seeberger, P. H. (2006) Miniaturization of microwave-assisted carbohydrate functionalization to create oligosaccharide microarrays. *ChemBioChem. 7*, 421−424.

(26) Bejugam, M., and Flitsch, S. L. (2004) An efficient synthetic route to glycoamino acid building blocks for glycopeptides synthesis. *Org. Lett. 6* (22), 4001−4004.

(27) Yates, E. A., Jones, M. O., Clarke, C. E., Powell, A. K., Johnson, S. R., Porch, A., Edwards, P. P., and Turnbull, J. E. (2003) Microwave enhanced reaction of carbohydrates with amino-derivatised labels and glass surfaces. *J. Mater. Chem. 13*, 2061−2063.

(28) Zhou, X., Zhang, J., and Wang, D. (2012) Microwave-assisted method for fabrication of carbohydrate cluster microarrays on 3-dimensional hydrazide-dendrimer substrate. *Carbohydrate Microarrays. Methods and Protocols. (Chevelot Ed.)* pp 401−411, Chapter 27, Humana Press, New York.

(29) Bouillon, C., Meyer, A., Vidal, S., Jochum, A., Chevolot, Y., Cloarec, J. P., Praly, J. P., Vasseur, J. J., and Morvan, F. (2006) Microwave assisted "click" chemistry for the synthesis of multiple labeled-carbohydrate oligonucleotides on solid support. *J. Org. Chem. 71*, 4700−4702.

(30) Ralet, M. C., Tranquet, O., Poulain, D., Moïse, A., and Guillon, F. (2010) Monoclonal antibodies to rhamnogalacturonan I backbone. *Planta 231*, 1373−1383.

(31) Mercey, E., Sadir, R., Maillart, E., Roget, A., Baleux, F., Lortat-Jacob, H., and Livache, T. (2008) Polypyrrole oligosaccharide array and surface plasmon resonance imaging for the measurement of glycosaminoglycan binding interactions. *Anal. Chem. 80*, 3476−3482.

(32) Ropartz, D., Bodet, P. E., Przybylski, C., Gonnet, F., Daniel, R., Fer, M., Helbert, W., Bertrand, D., and Rogniaux, H. (2011) Performance evaluation on a wide set of matrix-assisted laser desorption ionization matrices for the detection of oligosaccharides in a high-throughput mass spectrometric screening of carbohydrate depolymerizing enzymes. *Rapid Commun. Mass Spectrom. 25*, 2059−2070.

(33) Fiche, J. B., Fuchs, J., Buhot, A., Calemczuk, R., and Livache (2008) Point mutation detection bu surface plasmon resonance imaging coupled with a temperature scan method in a model system. T. *Anal. Chem. 80*, 1049−1057.

(34) Pagnotta, M., Pooley, C. L. F., Gurland, F., and Choi, M. (1993) Microwave activation of the mutarotation of α-D-glucose: An example of an interinsic microwave effect. *J. Phys. Org. Chem. 6*, 407−411.

(35) Gudmundsdottir, A. V., Paul, C. E., and Nitz, M. (2009) Stability studies of hydrazide and hydroxylamine-based glycoconjugates in aqueous solution. *Carbohydr. Res. 344*, 278−284.