Azide-Functionalized Derivatives of the Virulence-Associated Sugar Pseudaminic Acid: Chiral Pool Synthesis and Labeling of Bacteria

Amol M. Vibhute,[a] Hideki Tamai,[a] Dana Logviniuk,[b] Peter G. Jones,[c] Micha Fridman,*[b] and Daniel B. Werz*[a]

Abstract: Pseudaminic acid (Pse) is a significant prokaryotic monosaccharide found in important Gram-negative and Gram-positive bacteria. This unique sugar serves as a component of cell-surface-associated glycans or glycoproteins and is associated with their virulence. We report the synthesis of azidoacetamido-functionalized Pse derivatives as part of a search for Pse-derived metabolic labeling reagents. The synthesis was initiated with D-glucose (Glc), which served as a cost-effective chiral pool starting material. Key synthetic steps involve the conversion of C1 of Glc into the terminal methyl group of Pse, and inverting deoxyaminations at C3 and C5 of Glc followed by backbone elongation with a three-carbon unit using the Barbier reaction. Metabolic labeling experiments revealed that, of the four Pse derivatives, ester-protected C5 azidoacetamido-Pse successfully labeled cells of Pse-expressing Gram-positive and Gram-negative strains. No labeling was observed in cells of non-Pse-expressing strains. The ester-protected and C5 azidoacetamido-functionalized Pse is thus a useful reagent for the identification of bacteria expressing this unique virulence-associated nonulosonic acid.

Introduction

Whereas mammalian carbohydrates are generated from as few as ten different monosaccharides,[1] the diversity of glycans within bacteria is far greater.[2–5] Many monosaccharides of the prokaryotic kingdom differ structurally from their mammalian counterparts with regard to stereocentres, number of carbons, number of amino groups and/or in their decoration with unique chemical functionalities. One of the unusual sugars belonging to the family of nonulosonic acids is pseudaminic acid (Pse). The Pse unit is found in many glycans of both pathogenic and non-pathogenic bacteria.[6] In several cases Pse is part of an oligosaccharide;[7] however, it can be attached directly to a target protein (Figure 1).[8] Pse residues are found in the flagellar protein flagellin, in lipopolysaccharides and/or in capsules of *Pseudomonas aeruginosa*, *Vibrio vulnificus*, *Plesiomonas shigelloides*, *Bacillus thuringiensis* and *Campylobacter jejuni*.[7,8] Both axial (α) and equatorial (β) glycosidic linkages of Pse to a variety of pyranoses and furanoses,[7] and also to the alcohol functionalities of serine and threonine, have been characterized.[8] Although the structure of Pse was first elucidated in 1984 by Knirel and co-workers,[9] it was only in 2001 that the first chemical synthesis of Pse from 3,4-dibenzoyl rhamnose was published by Knirel, Zähringer and co-workers (Scheme 1).[10]

[a] *Dr. A. M. Vibhute, Dr. H. Tamai, Prof. Dr. D. B. Werz*
Technische Universität Braunschweig
Institute of Organic Chemistry, Hagenring 30
38106 Braunschweig (Germany)
E-mail: d.werz@tu-braunschweig.de
Homepage: www.werzlab.de

[b] *D. Logviniuk, Prof. Dr. M. Fridman*
School of Chemistry,
Raymond and Beverley Sackler Faculty of Exact Sciences
Tel Aviv University,
6997801 Tel Aviv (Israel)
E-mail: mfridman@tauex.tau.ac.il
Homepage: https://www.fridman-lab.sites.tau.ac.il/

[c] *Prof. Dr. P. G. Jones*
Technische Universität Braunschweig,
Institute of Inorganic and Analytical Chemistry
Hagenring 30, 38106 Braunschweig (Germany)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/chem.202100443

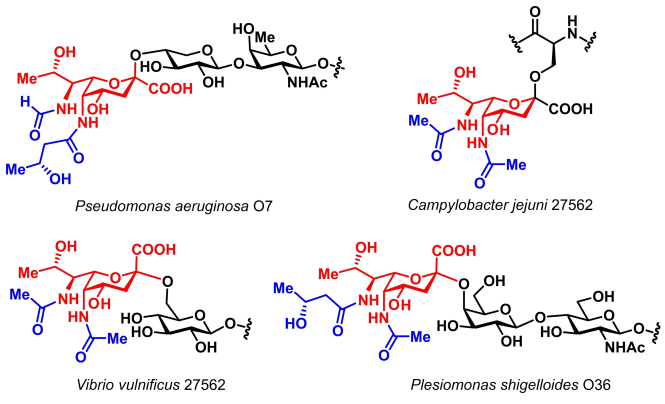
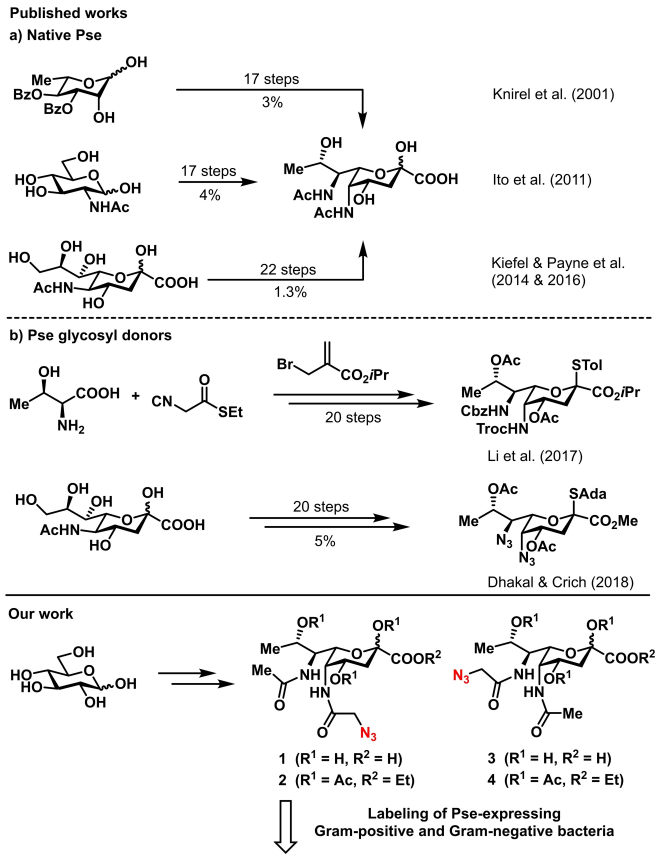


Figure 1. Examples of structures with pseudaminic acid (in red) from various bacteria.



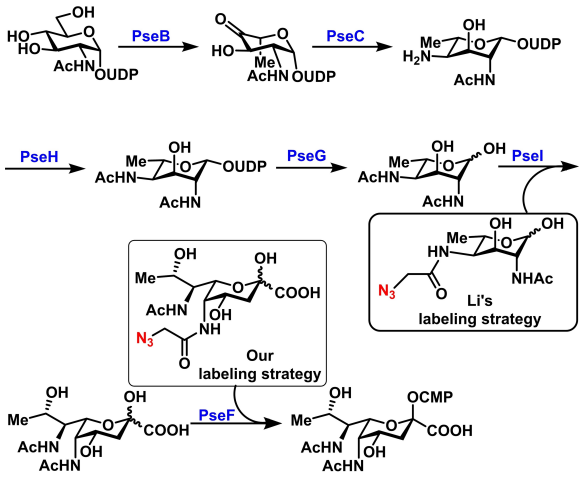
Scheme 1. Synthetic routes to native Pse and Pse glycosyl donors and our synthetic and biological work.

However, this synthetic route provided only small amounts of the *N*,*N*-diacetyl Pse congener.

In 2011 Ito and co-workers reported an alternative synthetic approach to diacetamide-functionalized Pse starting from *N*-acetylglucosamine.[11] In 2016 the Payne group reported the use of *N*-acetylneuraminic acid as the precursor for Pse synthesis.[12] In 2017, Li and co-workers presented a unique *de-novo* approach starting from L-threonine that allowed the synthesis of an appropriate Pse glycosyl donor, paving the way to the assembly of the trisaccharide found in the pilins of *P. aeruginosa* (Figure 1).[13] A similar approach had previously been described by Seeberger to access the related legionaminic acid.[14] Finally, in 2018, Crich and co-workers reported the conversion of *N*-acetylneuraminic acid to a Pse glycosyl donor and investigated the effects of the side chain conformation on the anomeric ratio of glycosylation with Pse glycosyl donors.[15] Since Pse derivatives isolated from different organisms often carry different functionalities at their N5 and N7 amines,[13] orthogonal amine-protecting groups or the selective transformation of azide functionalities are still required to reach the desired derivative, but affect the reactivity of the pyranose.[13,15]

Several glycoconjugates containing a number of Pse derivatives that differ in their N5 or N7 substitutions have been identified to date in various drug-resistant pathogens, and a connection between the Pse and virulence has been demonstrated.[16,17] Chemical probes for identification of Pse-expressing bacteria are therefore highly appealing and can pave the way for anti-virulence agents that inhibit Pse biosynthesis.

Li and co-workers reported the synthesis of 2-acetamido-4-azidoacetamido-2,4,6-trideoxy-L-altrose, an azidoacetamide-functionalized intermediate of the Pse biosynthetic pathway (Scheme 2).[18,19] This sugar was *O*-acetylated to facilitate passive membrane diffusion and was then used for metabolic labeling of Pse-expressing Gram-negative pathogens, including strains of *Pseudomonas aeruginosa, Vibrio vulnificus*, and *Acinetobacter baumannii*. Once inside the bacterial cell, azidoacetamide-functionalized trideoxy-L-altrose must go through four biosynthetic processes to be incorporated successfully into cell-surface Pse-containing oligosaccharides. Initially, the *O*-acetyl groups are removed by esterases, followed by Pse-synthase-catalyzed condensation with phosphoenolpyruvate to form the azidoacetamide-functionalized Pse. In the third step, the anomeric position of the azidoacetamide-functionalized Pse undergoes enzyme-catalyzed activation to form the CMP-sugar, and the final process is glycosyltransferase-catalyzed glycosylation onto the target cell surface oligosaccharides. We reasoned that placing the azidoacetamide functionality directly onto Pse should prove useful for metabolic incorporation in Pse-expressing bacteria, excluding the need for transformation of trideoxy-L-altrose to Pse catalyzed by Pse-synthase. Herein, we report on a straightforward and high-yielding chiral pool approach for the synthesis of azidoacetamide-functionalized Pse derivatives starting from D-glucose and their evaluation as metabolic labeling agents for detection of Pse-expressing bacteria (Scheme 1 and Scheme 2).

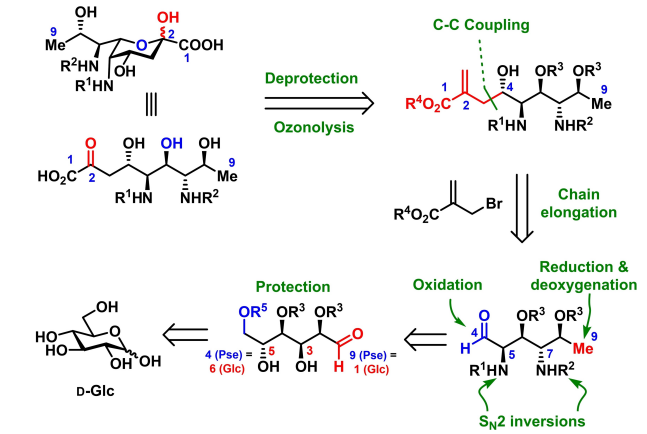


Scheme 2. CMP-pseudaminic acid biosynthetic pathway of *C. jejuni* and *H. pylori*,[19] Li's and our labeling pathways.

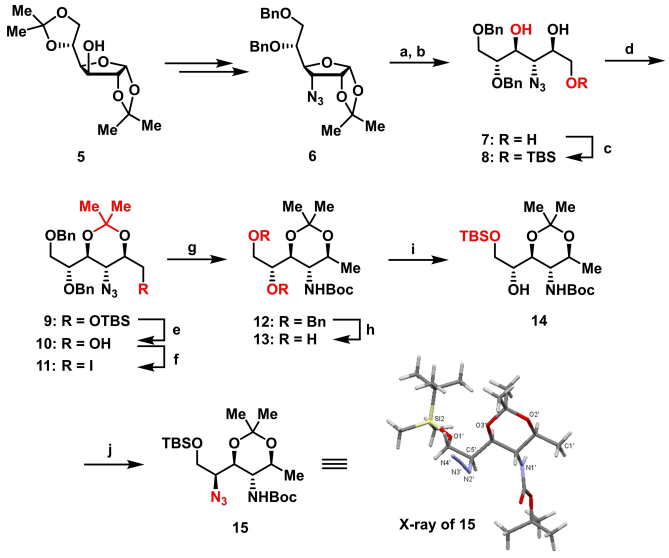
Results and Discussion

Retrosynthetic analysis

Whereas Ito's synthesis of Pse is derived from *N*-acetylglucosamine, whereby one of the two amino groups is functionalized as an acetamide,[11] we envisioned a chiral pool access starting from D-glucose. Drawing the cyclic hemiacetal with two differently protected amino groups (R^1, R^2) in its open form shows that the crucial keto moiety might be available by ozonolysis from an olefin (Scheme 3). We reasoned that introduction of the corresponding C3 unit to the hexose would be accomplished by the Barbier reaction.[20] The necessary C6 aldehyde can be obtained by reducing the anomeric carbon (C1 of Glc) to the corresponding methyl group (C9 of Pse) whereas the primary carbon (C6 of Glc) will be oxidized (C4 of Pse). The alcohols at the carbons 3 and 5 of glucose have to be replaced by (masked) amino groups, while the absolute configurations at these centers need to be reversed using S_N2-type nucleophilic substitutions (C5 and C7 of Pse).



Scheme 3. Retrosynthetic analysis of Pse with different amino residues.

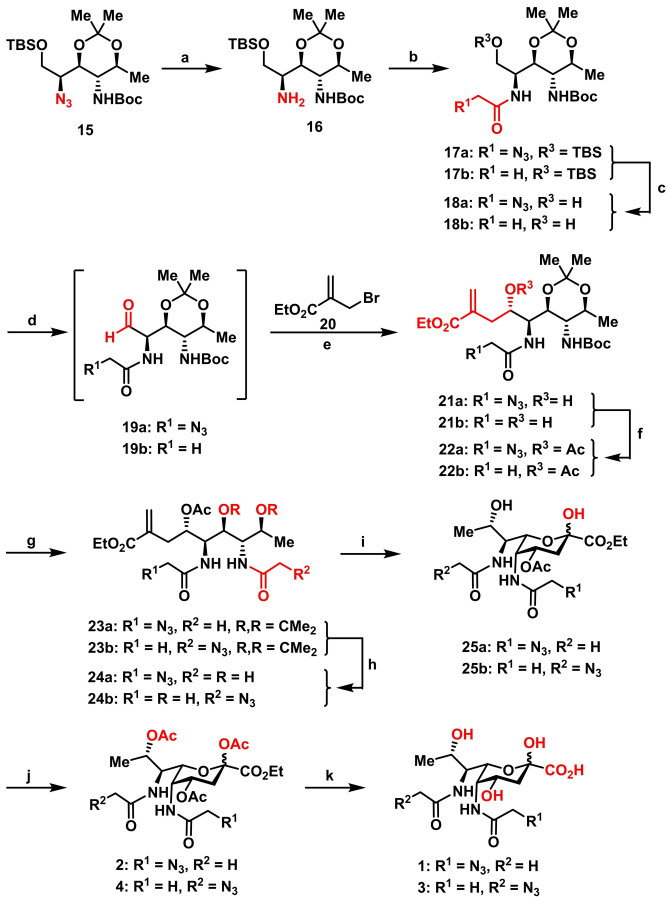


Scheme 4. Synthesis of intermediate **15**. Reagents and conditions: (a) AcOH (60% aq), 110 °C, 1 h; (b) $NaBH_4$, EtOH, rt, 48 h, 74% over 2 steps; (c) TBSCl, Et_3N, DMAP, DCM, rt, 12 h, 88%; (d) 2,2-dimethoxypropane, *p*-TSA, MeCN, rt, 1 h; (e) TBAF, rt, 12 h, 88% over 2 steps; (f) PPh_3, imidazole, I_2, MeCN, 70 °C, 1 h, 80%; (g) $^nBu_3SnH$, AIBN, Boc_2O, toluene, 100 °C, 1 h, 90%; (h) $Pd(OH)_2/C$, H_2, EtOH, rt, 12 h, 94%; (i) TBSCl, Et_3N, DMAP, DCM, rt, 12 h, 79%; (j) DEAD, DPPA, PPh_3, THF, rt, 12 h, 80%.

Synthesis of azidoacetamido-equipped Pse derivatives

We commenced the synthesis of azidoacetamido Pse derivatives from the commercially available starting material diisopropylidene-protected glucofuranose **5**.[21] The C3 alcohol of furanose **5** was substituted by an azide (Scheme 4).[22] Selective removal of the isopropylidene from C5 and C6 of **5** followed by bis-benzylation afforded **6**. The 1,2-isopropylidene was then cleaved under more vigorous acidic conditions followed by reduction using sodium borohydride to obtain open-chain triol **7**, whose primary alcohol was selectively protected with TBS. The remaining two secondary alcohols of **8** were suppressed with isopropylidene to furnish fully protected precursor **9**. To deoxygenate the C9 carbon of Pse, the TBS of **9** was removed with TBAF to form intermediate **10**. The free alcohol of **10** was then converted to the corresponding iodide **11** using Appel conditions. Compound **11** was subjected to reductive removal of iodide using stannane and AIBN as catalyst. Under these conditions the azide moiety is also able to undergo reduction to the corresponding amine; the resulting amine was trapped with a suitable protecting group. Interestingly, when the reaction was carried out in the presence of Boc anhydride, the fully protected compound **12** was obtained in 90% isolated yield. Next, the two adjacent benzyl ethers of **12** were selectively cleaved employing Pearlman's catalyst in the presence of hydrogen gas, affording diol **13**. The more accessible primary alcohol of **13** was next protected by TBS to yield compound **14**, paving the way for deoxyamination. To ensure inversion of configuration, we applied the Mitsunobu reaction with azide as a nucleophile. Diphenyl phosphoryl azide (DPPA) in presence of diethyl azodicarboxylate (DEAD) and triphenyl phosphine proved optimal and gave azide **15** in 80% yield (see Supporting Information). The structure of **15** was confirmed by X-ray analysis.

Azide **15** is still a C_6 chain; to reach the C_9 chain of Pse, a chain elongation by three carbon atoms is required. Initial attempts to cleave the TBS group in **15** and to oxidize the emerging primary alcohol to the corresponding aldehyde were in vain because α-azido aldehydes tend to decompose to nitriles shortened by one carbon. In order to circumvent this drawback, the azido unit was next reduced and the resulting amine coupled with 2-azido acetic acid in the presence of HATU to obtain azidoacetamide **17a** in 92% yield (Scheme 5). To obtain the acetyl-protected congener **17b**, common acylation conditions using acetic anhydride were employed. The TBS ether of **17a/17b** was removed with TBAF to yield **18a/18b**, which were oxidized to the corresponding aldehydes **19a** and **19b**, respectively, using IBX. Chain elongation of these capricious intermediates was accomplished via the Barbier reaction.



Scheme 5. Synthesis of the target compounds **1**–**4**: Reagents and conditions: (a) PPh_3, THF, H_2O, reflux 3 h, 88%; (b) For **17a**: HATU, 2-azido acetic acid, DIPEA, THF:DMF (3:2, v/v), rt, 3 h, 92%; for **17b**: Ac_2O, Et_3N, DCM, rt, 2 h, 74%; (c) TBAF, THF, rt, 30 min, **18a**: 74%, **18b**: 99%; (d) IBX, DMSO, rt, 12 h; (e) In, THF/H_2O, Sonication, 50 °C, 1 h, then rt, 2 h, **21a**: 56% (over 2 steps), **21b**: 46% (over 2 steps); (f) Ac_2O, DMAP, pyridine, 0 °C to rt, 2 h, **22a**: 81%, **22b**: 80%; (g) Me_3SiI, DCM, rt, 10 min; for **23a**: Ac_2O, Et_3N, DCM, rt, 1 h, 87% (over 2 steps); for **23b**: HATU, 2-azido acetic acid, DIPEA, THF:DMF (3:2, v/v), rt, 3 h, 99% (over 2 steps); (h) AcOH (60% aq), 50 °C, 24 h, **24a**: 81%, **24b**: 67%; (i) O_3, DCM, −78 °C, 45 min, Me_2S, rt, 1 h, **25a**: 98%, **25b**: 89%; (j) Ac_2O, pyridine, DMAP, 0 °C to rt, 2 h, **2**: 94%, **4**: 86%; (k) Et_3N, H_2O, rt, 2 h, **1**: 59%, **3**: 54%.

Briefly, indium and 2-bromomethyl ethyl acrylate (**20**) were sonicated to form a bromoindium complex.[14] Careful addition of this reactive species to a solution of the respective aldehyde **19a/19b** in THF gave the desired *anti*-configured compounds **21a/21b** together with their *syn*-diastereoisomers in a ratio of 3:1 (Supporting Information). The two diastereoisomers were readily separable by flash chromatography. The newly generated alcohol groups were protected by acetates and the Boc group was cleaved by trimethylsilyl iodide.[23] The emerging amines were then acylated under the conditions mentioned above, furnishing diamides **23a** and **23b**, respectively. The isopropylidene was next removed in aqueous acetic acid, affording diols **24a** and **24b**, which were then subjected to ozonolysis. The scission of the olefinic entities led to highly reactive keto functionalities that were immediately attacked by an alcohol, leading to the cyclized Pse derivatives **25a/25b**. To obtain the more lipophilic, completely protected, azido-labeled Pse derivatives, acylation with acetic anhydride in the presence of pyridine gave the target compounds **2** and **4** in good yield. In order to obtain native azidoacetamido Pse derivatives **1** and **3**, the acetate protection was removed using triethylamine-mediated hydrolysis to afford the target compounds as triethylammonium salts (Scheme 5).

Metabolic labeling of bacteria with azidoacetamide-functionalized Pse derivatives 1–4

To establish whether azidoacetamide-functionalized pseudaminic acid derivatives **1** and **3** or their ester-protected derivatives **2** and **4**, respectively, can be used as metabolic labeling reporters for Pse-expressing bacteria, we studied their incorporation in two Pse-expressing bacteria: Gram-positive *Bacillus thuringiensis Berliner* ATCC strain 35646 and Gram-negative *Campylobacter jejuni* ATCC strain BAA-2151. As negative controls, we tested possible incorporation of Pse derivatives **1**–**4** in the Gram-positive *Staphylococcus aureus* ATCC strain 29213 and the Gram-negative *Escherichia coli* ATCC strain 25922, which do not express Pse. It is noteworthy that Pse was first identified in several Gram-negative pathogens. As a result, the Pse biosynthetic pathway has not been as well characterized in Gram-positive bacteria. Optimized labeling conditions for each bacterium are detailed in the Supporting Information. Briefly, *B. thuringiensis, S. aureus* and *E. coli* cultures were incubated with 2.5 mM of the azido-functionalized Pse derivatives for 1 h, whereas *C. jejuni* required 12 h of incubation under anaerobic conditions. The cultures were then washed and labeled with the fluorescent dye TAMRA-PEG4-DBCO via copper-free click reaction with the azido sugars, after which the cells were washed with PBS buffer to minimize non-specific staining and imaged by fluorescence microscopy (Figure 2).

Only the N5-azidoacetamide and ester-protected Pse derivative **2** clearly labeled both of the Pse-expressing bacterial strains. In contrast no labeling was observed for the corresponding deprotected N5-azidoacetamide Pse **1** indicating that the uptake of Pse derivatives probably occurs via passive diffusion through the membrane rather than by a transporter or anion channel. Pse derivatives **2** and **4** differ solely in the position of the azidoacetamide functionality (N5 or N7, respectively), yet no labeling was observed for bacteria incubated with the latter. The reason might be either that the presence of an acetamide at N7 may impair substrate recognition by enzymes involved in the incorporation of this Pse derivative or that the N7-acetamide is further metabolized by the tested bacteria. Notably, of the two azidoacetamido-functionalized 2,4,6-trideoxy-L-altrose derivatives synthesized by Li and coworkers, the 4-azidoacetamido derivative, which is a precursor for N7-azidoacetamide Pse, was successfully metabolized into Pse while 2-azidoacetamido functionalized L-altrose, which is a precursor for N5-azidoacetamide Pse, failed and did not label the bacteria. These differences might be reasoned by differences in the substrate specificity of the enzymes of the Pse metabolic pathway involved in the metabolism of Li's

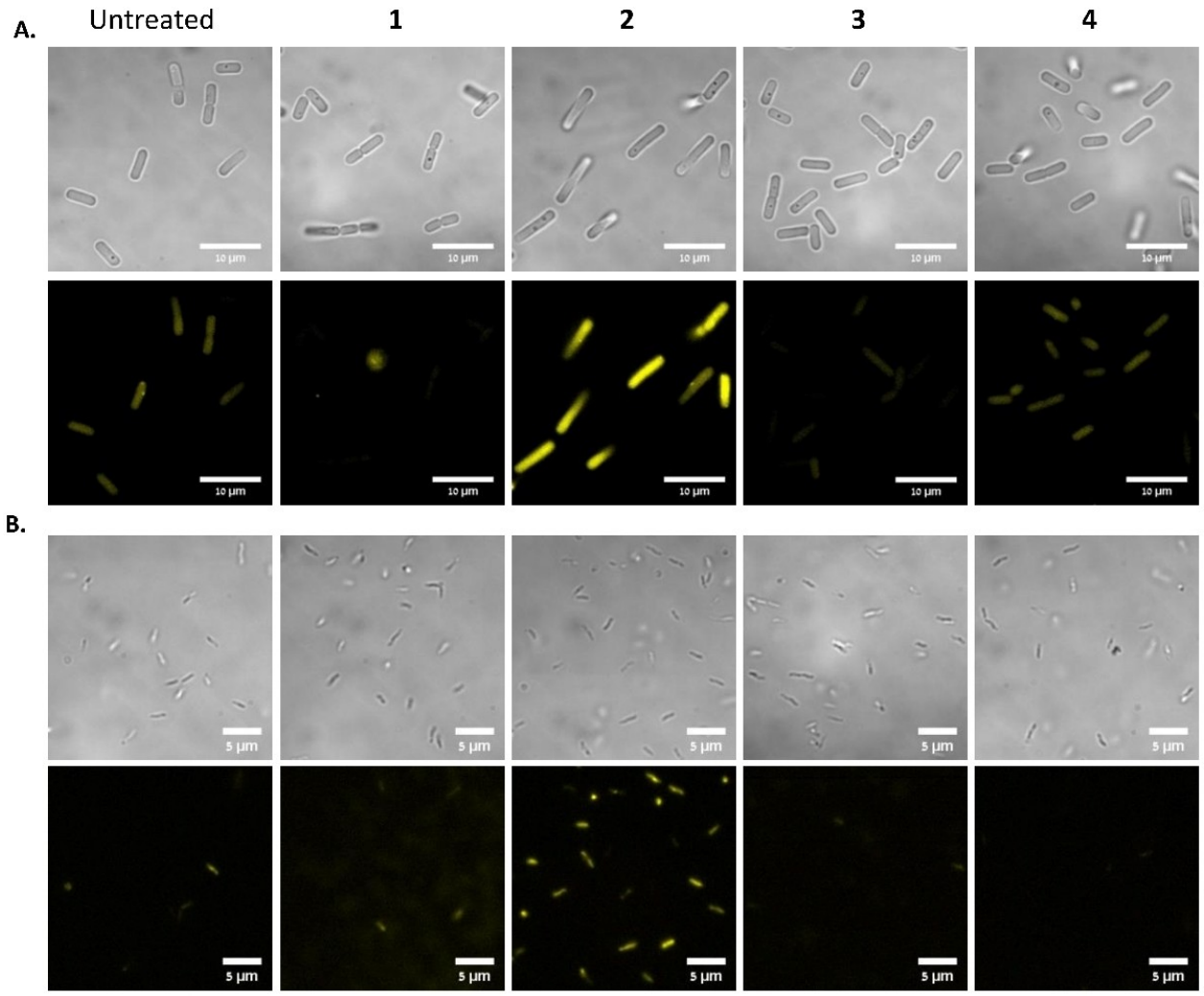


Figure 2. Metabolic labeling with Pse derivatives **1**–**4**: Bright field images (top) and fluorescence images of: A) *Bacillus thuringiensis* ATCC 35646 (scale bars 10 µm); B) *Campylobacter jejuni* ATCC BAA-2151 (scale bars 5 µm). The bandpass filters used for the fluorescent images had an excitation wavelength of 537/26 nm and an emission wavelength of 607/36 nm.

azidoacetamido-functionalized L-altrose derivatives and our Pse derivatives.

Importantly, N5 azidoacetamido Pse derivative **2** did not label cells of non-Pse expressing *S. aureus* and *E. coli* strains, which were tested as negative controls (see Supporting Information). These results support the view that ester-protected N5-azidoacetamide Pse derivative **2** labels the bacterial cells via the Pse pathway and not via an alternative metabolic process. Lastly, depending on the bacteria, Pse was found as a component of lipopolysaccharide or the capsule or as a posttranslational modification of the flagellar protein flagellin. While Pse was reported to decorate flagellin in both *B. thuringiensis* and *C. jejuni*, the entire envelope treated with **2** was fluorescently labelled. This suggests that, in these bacteria, Pse may decorate additional sites and not only flagellin.

Conclusion

We report a straightforward route, starting from D-glucose, to access azidoacetamide-functionalized Pse derivatives. Inverting deoxyaminations of the C3 and C5 alcohols of Glc facilitated the installation of the nitrogen functionalities of Pse. The aldehyde of Glc was reduced to a methyl group and later became the C9 terminus of Pse, whereas the primary alcohol (C6 of Glc) was oxidized to an aldehyde. The latter functionality set the stage for chain elongation with a C_3 unit by the Barbier reaction using a bromoallyl fragment. Ozonolysis gave the keto functionality (C2 of Pse). Our synthetic strategy allows for selective functionalization of either of the two Pse amino groups and the installation of the desired acyl group to form either an acetamide or azidoacetamide. N5- and N7-azidoacetamide-functionalized congeners were obtained in lipophilic and hydrophilic forms (ester-protected or deprotected carbohydrate scaffolds, respectively).

The azidoacetamide-functionalized Pse substrates reported herein are useful biochemical and diagnostic molecular tools. Pse glycosyltransferases such as Maf1, involved in the transfer of Pse onto flagellin, were studied by an immunoblotting assay.[24] Access to azidoacetamide-functionalized Pse substrates should prove useful for identification and studies of the kinetic parameters of Pse glycosyltransferases and for the identification of their protein target(s). We applied azidoacetamide-functionalized Pse for detecting Pse-expressing bacteria. Metabolic labeling experiments on *B. thuringiensis* and *C. jejuni*, Pse-expressing Gram-positive and Gram-negative bacteria, indicated that N5-azidoacetamide labeled Pse **2** is a useful indicator for the presence of the Pse pathway in bacteria. Since Pse expression was associated with virulence, metabolic labeling with Pse derivative **2** offers a useful and robust assay for detection of virulence in Pse-expressing bacteria and for identification of anti-virulence agents that act by targeting the Pse biosynthetic pathway.

Experimental Section

Crystallographic data

Deposition Number 2058563 contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

Acknowledgements

We are grateful to the TU Braunschweig for funding. H.T. thanks the Alexander von Humboldt Foundation for a postdoctoral fellowship. Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: azidoacetamide · bacteria · chiral pool · metabolic labeling · pseudaminic acid

[1] D. B. Werz, R. Ranzinger, S. Herget, A. Adibekian, C.-W. von der Lieth, P. H. Seeberger, *ACS Chem. Biol.* **2007**, *2*, 685–691.
[2] A. Adibekian, P. Stallforth, M.-L. Hecht, D. B. Werz, P. Gagneux, P. H. Seeberger, *Chem. Sci.* **2011**, *2*, 337–344.
[3] S. I. Elshahawi, K. A. Shaaban, M. K. Kharel, J. S. Thorson, *Chem. Soc. Rev.* **2015**, *44*, 7591–7697.
[4] a) A. P. Moran, O. Holst, P. J. Brennan, M. von Itzstein (Eds.), *Microbial Glycobiology*, Elsevier: Amsterdam, **2009**; b) P. Kosma, *Curr. Org. Chem.* **2008**, *12*, 1021–1039; c) Y. A. Knirel, A. S. Shashkov, Y. E. Tsvetkov, P.-E. Jansson, U. Zähringer, *Adv. Carbohydr. Chem. Biochem.* **2003**, *58*, 371–417.
[5] For some syntheses of bacterial mono- and oligosaccharides: a) S. R. Sanapala, S. S. Kulkarni, *J. Am. Chem. Soc.* **2016**, *138*, 4938–4947; b) B. Mishra, M. Neralkar, S. Hotha, *Angew. Chem.* **2016**, *128*, 7917–7922; *Angew. Chem. Int. Ed.* **2016**, *55*, 7786–7791; c) M. Islam, G. P. Shinde, S. Hotha, *Chem. Sci.* **2017**, *8*, 2033–2038; d) A. Behera, D. Rai, S. S. Kulkarni, *J. Am. Chem. Soc.* **2019**, *142*, 456–467; e) S. Ahadi, S. I. Awan, D. B. Werz, *Chem. Eur. J.* **2020**, *26*, 6264–6270; f) M. A. Oberli, P. Bindschädler, D. B. Werz, P. H. Seeberger, *Org. Lett.* **2008**, *10*, 905–908; g) D. B. Werz, P. H. Seeberger, *Angew. Chem.* **2005**, *117*, 6474–6476; *Angew. Chem. Int. Ed.* **2005**, *44*, 6315–6318.
[6] M. Zunk, M. J. Kiefel, *RSC Adv.* **2014**, *4*, 3413–3421.
[7] Representative examples: a) S. N. Senchenkova, A. V. Popova, A. S. Shashkov, M. M. Shneider, Z. Mei, N. P. Arbatsky, B. Liu, K. A. Miroshnikov, N. V. Volozhantsev, Y. A. Knirel, *Carbohydr. Res.* **2015**, *407*, 154–157; b) E. Vinogradov, M. Frimmelova, R. Toman, *Carbohydr. Res.* **2013**, *378*, 108–113; c) J. Muldoon, A. S. Shashkov, S. N. Senchenkova, S. V. Tomshich, N. A. Komandrova, L. A. Romanenko, Y. A. Knirel, A. V. Savage, *Carbohydr. Res.* **2001**, *330*, 231–239; d) P. Castric, F. J. Cassels, R. W. Carlson, *J. Biol. Chem.* **2001**, *276*, 26479–26485; e) M. Kaszowska, K. Stojkovic, T. Niedziela, C. Lugowski, *Carbohydr. Res.* **2016**, *434*, 1–5; f) J. J. Kenyon, A. M. Marzaioli, R. M. Hall, C. De Castro, *Carbohydr. Res.* **2015**, *409*, 30–35; g) A. V. Perepelov, A. S. Shashkov, V. I. Torgov, E. L. Nazarenko, R. P. Gorshkova, E. P. Ivanova, N. M. Gorshkova, G. Widmalm, *Carbohydr. Res.* **2005**, *340*, 69–74.
[8] a) P. Thibault, S. M. Logan, J. F. Kelly, J.-R. Brisson, C. P. Ewing, T. J. Trust, P. Guerry, *J. Biol. Chem.* **2001**, *276*, 34862–34870; b) Y. A. Knirel, O. V. Bystrova, N. A. Kocharova, U. Zähringer, G. B. Pier, *J. Endotoxin Res.* **2006**, *12*, 324–336.
[9] Y. A. Knirel, E. V. Vinogradov, V. L. L'vov, N. A. Kocharova, A. S. Shashkov, B. A. Dmitriev, N. K. Kochetkov, *Carbohydr. Res.* **1984**, *133*, C5–C8.
[10] Y. E. Tsvetkov, A. S. Shashkov, Y. A. Knirel, U. Zähringer, *Carbohydr. Res.* **2001**, *335*, 221–243.
[11] Y. J. Lee, A. Kubota, A. Ishiwata, Y. Ito, *Tetrahedron Lett.* **2011**, *52*, 418–421.
[12] a) J. T. Williams, L. Corcilius, M. J. Kiefel, R. J. Payne, *J. Org. Chem.* **2016**, *81*, 2607–2611; b) M. Zunk, J. Williams, J. Carter, M. J. Kiefel, *Org. Biomol. Chem.* **2014**, *12*, 2918–2925.
[13] a) H. Liu, Y. Zhang, R. Wei, G. Andolina, X. Li, *J. Am. Chem. Soc.* **2017**, *139*, 13420–13428; b) R. Wei, H. Liu, A. H. Tang, R. J. Payne, X. Li, *Org. Lett.* **2019**, *21*, 3584–3588.
[14] S. Matthies, P. Stallforth, P. H. Seeberger, *J. Am. Chem. Soc.* **2015**, *137*, 2848–2851.
[15] a) B. Dhakal, D. Crich, *J. Am. Chem. Soc.* **2018**, *140*, 15008–15015; b) B. Dhakal, S. Buda, D. Crich, *J. Org. Chem.* **2016**, *81*, 10617–10630.
[16] E. K. P. Flack, H. S. Chidwick, G. Guchhait, T. Keenan, D. Budhadev, K. Huang, P. Both, J. Mas Pons, H. Ledru, S. Rui et al. *ACS Catal.* **2020**, *10*, 9986–9993.
[17] R. Ménard, I. C. Schoenhofen, L. Tao, A. Aubry, P. Bouchard, C. W. Reid, P. Lachance, S. M. Twine, K. M. Fulton, Q. Cui, et al. *Antimicrob. Agents Chemother.* **2014**, *58*, 7430–7440.
[18] G. Andolina, R. Wei, H. Liu, Q. Zhang, X. Yang, H. Cao, S. Chen, A. Yan, X. D. Liu, X. Li, *ACS Chem. Biol.* **2018**, *13*, 3030–3037.
[19] I. C. Schoenhofen, D. J. McNally, J. R. Brisson, S. M. Logan, *Glycobiology* **2006**, *16*, 8C-14C.
[20] C. Blomberg, F. A. Hartog, *Synthesis* **1977**, 18–30.
[21] H. van Grunenberg, C. Bredt, W. Freudenberg, *J. Am. Chem. Soc.* **1938**, *60*, 1507.
[22] S. A. W. Gruner, V. Truffault, G. Voll, E. Locardi, M. Stockle, H. Kessler, *Chem. Eur. J.* **2002**, *8*, 4365–4376.
[23] R. S. Lott, V. S. Chauhan, C. H. Stammer, *J. Chem. Soc. Chem. Commun.* **1979**, 495.
[24] J. L. Parker, R. C. Lowry, N. A. S. Couto, P. C. Wright, G. P. Stafford, J. G. Shaw, *Mol. Microbiol.* **2014**, *92*, 258–272.

Manuscript received: February 4, 2021
Accepted manuscript online: March 26, 2021
Version of record online: May 2, 2021

