Società Chimica Italiana

Gruppo Interdivisionale
Chimica dei Carboidrati

ATTI XVI CONVEGNO-SCUOLA SULLA CHIMICA DEI CARBOIDRATI

17-20 Giugno 2018
Certosa di Pontignano – Siena

Università di Pisa
Dipartimento di Farmacia

Università di Siena
Dipartimento di Biotecnologie, Chimica e Farmacia

Università di Firenze
Dipartimento di Chimica "U. Shiff"

COMITATO SCIENTIFICO:

Antonio MOLINARO	Università di Napoli (*Coordinatore GICC*)
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Cristina NATIVI	Università di Firenze
Antonella Bisio	IRCB-Ronzoni, Milano
Paola Cescutti	Università di Trieste
Francesco Berti	GSK Vaccines, Siena

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Roberta MARCHETTI	Università di Napoli
Francesco GIORGELLI	Università di Pisa
Maurizio BOTTA	Università di Siena

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SEDE DEL CONVEGNO

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Il Comitato Scientifico e il Comitato Organizzatore ringraziano:

http://www.labservice.it

http://www.bruker.com

http://www.giottobiotech.com

http://www.loghia.com

www.biotage.com

XVI CSCC Program

	Sunday June 17th
11:00÷16:00	**Registration**
16:00÷16:30	**Opening** *Prof. Gianluca Farinola (Presidente della Divisione della Chimica Organica –S.C.I.);* *Prof. Maria Rosaria Tiné ((Presidente della sezione Toscana della SCI.)* *Prof. Andrea Goti (Direttore del Dipartimento di Chimica "Ugo Schiff, Università di Firenze)*
	Chairman: DOMENICO GAROZZO
16:30÷17:15	**"G. Berti" Lecture: Paolo Costantino** (*GSK Vaccines S.r.l., Siena*) **Glycoconjugate vaccines: lessons learned and new trend**
	Chairman: FRANCESCO NICOTRA
17.15÷18.00	**PL-1: Biao Yu** (*Shanghai Institute of Organic Chemistry - Shanghai- Cina*) **Chemical Synthesis of Marine Saponins**
18:00÷18:45	**PL-2: Claudia Bello** (*Università di Firenze*) **An Auxiliary-Mediated Approach for the Simple Preparation of Complex Glycopeptides and Glycoproteins.**
19:30	**Welcome Cocktail**
	Monday June 18th Morning
	Chairman: LUIGI LAY
9:00÷9:50	**DL-1: Alba Silipo** (*Università di Napoli*) **Understanding the role of Glycoconjugates as key Molecules for Cell Survival and Communication in Prokaryotes and Eukaryotes**
9:50÷10:05	**OC-1: Martina Delbianco** (*Max Planck Institute of Colloids and Interfaces, Potsdam, Germany*) **Well-Defined Oligo and Polysaccharides as Ideal Probes for Structural Studies.**
10:05÷10:20	**OC-2: Sabrina Santarsia, Cristina Nativi, Filipa Marcelo, Ana Luísa Carvalho, Jesus Jiménez-Barbero, Francesco Papi** *(Università di Firenze)* **Synthesis and studies of TF tumor-associated antigen mimetic with Gal-3.**
10:20÷10:35	**OC-3: Giulia Risi, Sabrina Bertini, Elisa Masseroni, Marco Sansò** (*IRCB-Ronzoni, Milano)* **Characterization of Hyaluronic Acid-Tamarind Seeds Oftalmic Formulations**
10:35÷10:50	**OC-4: Noemi Veraldi, Marco Guerrini, Elena Urso, Giulia Risi, Sabrina Bertini, Antonella Bisio** (*IRCB-Ronzoni, Milano)* **Fine Structural Characterization of Sulodexide, a Heparinoid Drug**
10:50÷11:05	**OC-5: Filippo Carboni, Roberto Adamo, Monica Fabbrini, Riccardo De Ricco, Barbara Brogioni, Daniele Veggi, Vittoria Pinto, Davide Oldrini, Rino Rappuoli, Enrico Malito, Immaculada Margarity Ros, Francesco Berti.** (*GSK Vaccines S.r.l., Siena*) **Structure of a protective epitope of Group B Streptococcus type III capsular polysaccharide**
11:05÷11:30	***Coffee Break***

	Chairlady: CRISTINA DE CASTRO
11:30÷12:15	**PL-3: Manuel A. Coimbra** (*University of Aveiro - Portugal*) **Polysaccharides chemistry and food applications: from sulphur dioxide alternatives to acrylamide mitigation**
12:15÷12:30	**OC-6: Flaviana Di Lorenzo, Alba Silipo, Antonio Molinaro** (*Università di Napoli*) **Gut Microbiota Lipopolysaccharides: Reverting the Concept from Bad to Good**
12:30÷12:45	**OC-7: Mateusz Pallach, Flaviana Di Lorenzo, Fabio Facchini, Francesco Peri, Katarzyna A. Duda, Antonio Molinaro, Alba Silipo** (*Università di Napoli*) **Lipolysaccharides and the Innate Immunity: Structural and Immunostimulant Studies on *Acetobacter Pasteurianus* CIP103108 and *Phaeobacter Gallaeciensis* BS107 Lipopolysaccharide**
12:45÷13:00	**OC-8: Nicola Di Fidio, Sara Fulignati, Claudia Antonetti, Anna Maria Raspolli Galletti** (*Università di Pisa*) **Production of Second Generation Sugars from Giant Reed and Cardoon as Potential Feedstock for the Bioconversion into Microbial Lipids**
13:00÷14:30	***Lunch in Certosa***
	Monday June 18th Afternoon
	Chairlady: ANTONELLA BISIO
14:30÷14:45	**Premio intitolato alla memoria del Prof. Benito Casu**
14:45÷15:00	**OC-9: "B. Casu" Oral presentation:**
15:00÷15:15	**OC-10: Andrea Mezzetta, Lorenzo Guazzelli, Stefano Becherini, Cinzia Chiappe.** (*Università di Pisa*) **Address the challenge of the development of sustainable materials by exploiting ILs and polysaccharides**
15:15÷17:15	**POSTER SESSION AND *COFFEE BREAK* (16.00-16.30)**
17:15	***Meeting of the Italian Group on Carbohydrate Chemistry (GICC)***
20:00	***Dinner in Certosa***
	"Serge Perez" Session, chairman Antonio Molinaro
21:45-22:15	**DL-2: Serge Perez** (*CNRS – University of Grenoble Alpes*) **A computer tool box for glycoscience**
	Tuesday June 19th Morning
	Chairman: OSCAR FRANCESCONI
9:00÷9:50	**DL-3: Fabrizio Chiodo** (*Institute of Chemistry, Bio-Organic Synthesis - Netherlands*) **Design and Preparation of Multivalent Gold Nanoparticles to Interrogate and Investigate Immunological Properties of Carbohydrates**
9:50÷10:05	**OC-11: Olimpia Pitirollo, Martina Carducci, Francesca Mancini, Francesca Necchi, Laura Polito, Luigi Lay, Roberto Adamo, Francesca Micoli** (*Università di Milano*) **Synthesis of Hexarhamnose-CRM197 Conjugate and its Immunogenicity Against Group A Streptococcus Polysaccharide**

10:05÷10:20	**OC-12: Todaro Biagio, Laigre Eugénie, Liet Benjamin, Goyard David, Renaudet Olivier** (*UMR-CNRS, Grenoble, France*) **Engineering of Biomolecular Systems for Anti-Tumoral Immunotherapy**
10:20÷10:35	**OC-13: Renzo Alfini, Gianluigi De Benedetto, Roberta Di Benedetto, Maria Michelina Raso, Francesca Necchi, Gianmarco Gasperini, Paola Cescutti, Cario Giannelli, Allan Saul, Francesca Micoli** (*GSK Vaccines Institute for Global Health, Siena*) ***O*-antigen GMMA-based vaccine against nontyphoidal *Salmonella***
10:35÷10:50	**OC-14: Maria Grazia Ciampa, Pamela Fato, Sandro Sonnino, Laura Mauri** (*Università di Milano*) **Synthesis of II3Neu5Ac-[6-3HGal]Gg4-N3**
10:50÷11:20	***Coffee Break***
	Chairlady: ALBA SILIPO
11:20÷12:05	**PL-4: Christoph Rademacher** (*Max Planck Institute of Colloids and Interfaces, Potsdam, Germany*) **Fragment-based design of carbohydrate receptor ligands**
12:05÷12:20	**OC-15: Paul R. Crocker, Koichi Fukase, Yoshiyuki Manabe, Antonio Molinaro, Alba Silipo, Roberta Marchetti** *(Università di Napoli)* **Novel Insights into *N*-Glycans Recognition by Host Proteins**
12:20÷13:05	**PL-5: Sabrina Bertini** (*IRCB-Ronzoni, Milano*) **Chemical Physical Characterization of Polysaccharides and their Interaction with Proteins**
13:10÷14:30	***Lunch in Certosa***
	Tuesday June 19th Afternoon
	Chairman: GENNARO PICCIALLI
14:30-15:15	**PL-6: Laura Russo** (*Università di Milano-Bicocca*) **Glycans and Biomaterials: from Nanomedicine Applications to 3D Bioprinted Cell Culture Models**
15:15-15:30	**OC-16: Maria Michelina Raso, Gianmarco Gasperini, Paola Cescutti, Francesca Micoli** *(Università di Trieste and GSK Vaccines S.r.l., Siena)* ***O*-Antigen Characterization on Generalized Modules for Membrane Antigens (GMMA) from *Shigella Flexneri* 6**
16:00	**Social Event & Social dinner**
	Wednesday June 20th Morning
	Chairman: ANDREA GOTI
9.00÷9.45	**PL-7: Cinzia Colombo** (*Università di Milano*) **Strategies for the Synthesis of Uronic Acid Oligosaccharides. *Salmonella typhi Vi* Antigen as a Case Study**
9.45÷10.00	**OC-17: Costanza Vanni, Giampiero D'Adami, Matilde Forcella, Paola Fusi, Camilla Matassini, Xhenti Ferhati, Francesca Cardona** (*Università di Firenze*) **Iminosugar-Based Trehalase Inhibitors: the Key Role of Linker Length and Flexibility**
10.00÷10.15	**OC-18: Anna Esposito, Maria De Fenza, Daniele D'Alonzo, Annalisa Guaragna** (*Università di Napoli*) **De Novo Synthesis of *L*-DNJ and its *N*-Alkylated Derivatives**

10.15÷10.30	**OC-19: Serena Fortunato, Chiara Lenzi, Valeria Di Bussolo, Carlotta Granchi, Alma Martelli, Valentina Citi, Vincenzo Calderone, Filippo Minutolo.** (*Università di Pisa*) **Synthesis of Glycoconjugate H_2S-Donors**
10.30÷10.45	**OC-20: Alice Tamburrini, Nives Hribernik, Corinne Deniaud, Franck Fieschi, Anna Bernardi.** (*Università di Milano*) **One-pot Synthesis of Pseudo-thiodisaccharides through Aziridine Opening Reactions**
10.45÷11.15	***Coffee Break***
	Chairman: ROBERTO RIZZO
11.15÷11.30	**OC-21: Ludovic Auberger, Jacopo Enotarpi, Ilaria Calloni, Cristina Santi, Laura Polito, Jesús Jiménez-Barbero, Jeroen Codée, Roberto Adamo, Luigi Lay.** (*Università di Milano*) **Synthesis of MENA Hydrolytically Stable Analogues for Improved Anti Meningococcal Vaccine.**
11.30÷11.45	**OC-22: Aurora Mancuso, Paola Bonaccorsi, Tania M. G. Salerno, Anna Barattucci** (*Università di Messina*) **Design and Synthesis of Modified Glicoamino OPEs for the Finding of New Potential Photosensitizers**
11.45÷12.00	**OC-23: Giulia Vessella, Antonio Fabozzi, Angela Casillo, Caroline I. Biggs, Matthew I. Gibson, Maria Michela Corsaro, Emiliano Bedini** *(Università di Napoli)* **Synthesis and Ice-Recrystallization Inhibition Evaluation of the Tetrasaccharide Repeating Unit of the Cryoprotectant Capsular Polysaccharide from Colwellia psychrerythraea 34H**
12.15÷12.40	**Poster awards and young scientists oral communication awards**
12.40÷13.00	***Closing remarks***
13.00	***Lunch in Certosa***

PL: Plenary Lecture; DL: Didactic Lecture; OC: Oral Communication;

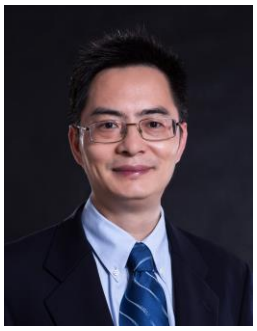
Poster Communications

Pilar García del Vello Moreno, Antonio Molinaro, Willem De Vos, **Cristina De Castro**. *(Università di Napoli)* **Decoding Akkermansia Muciniphila Lipopolysaccharides** **e-mail: pilar.garciadelvellomoreno@unina.it**	**PC-1**
Clara Barrau, Flaviana Di Lorenzo, Antonio Molinaro, **Alba Silipo** (*Università di Napoli*) **Structure of Lipooligosaccharides from Halophilic Bacteria** **e-mail: clarabarrau@gmail.com**	**PC-2**
Molly Pither, Flaviana Di Lorenzo, Alba Silipo, Mark Skidmore and Antonio Molinaro (*Università di Napoli*) **Chemical structure of lipopolysaccharides isolated from *veillonella parvula*** **e-mail: mollydotpither@hotmail.co.uk**	**PC-3**
Meriem Maaleja, Roberta Marchetti, Alba Silipo, Cédric Laguri, Jean-Pierre Simorre, Antonio Molinaro**.** (*Université Grenoble Alpes, Università di Napoli*) **Deciphering the recognition patterns of lectins interaction with glycan motifs of Lipopolysaccharides using Nuclear Magnetic Resonance (NMR)** **e-mail: meriem.maalej2@etu.unistra.fr**	**PC-4**
Gianluigi De Benedetto, Krisztina Hitri, Michelle Kuttel, Kay Lockyer, Fang Gao, Peter Hansal, Timothy R. Rudd, Sjoerd Rijpkema, Neil Ravenscroft, Barbara Bolgiano. (*NIBSC, Hertfordshire U.K.)* **Analysis of the Structural Basis of *O*-Acetyl Epitopes as Key Components of the Immunogenicity of VI polysaccharide** **e-mail: Gianluigi.DeBenedetto@nibsc.org**	**PC-5**
Maria Michelina Raso, Gianmarco Gasperini, Paola Cescutti, Francesca Micoli *(Università di trieste, GSK Vaccines S.r.l. Siena)* **O-Antigen Characterization on Generalized Modules for Membrane Antigens (GMMA) from *Shigella Flexneri* 6** **e-mail: mariamichelina.raso@phd.units.it**	**PC-6**
Barbara Bellich, Zois Syrgiannis, Roberto Rizzo, **Paola Cescutti** (*Università di Trieste*) **Atomic Force Microscopy Investigation of the Biofilm Polysaccharide from *Burkholderia Multivorans* C1576** **e-mail: pcescutti@units.it**	**PC-7**
Luigi Lay, **Cristina Manuela Santi** (*Università di Milano*) **Synthesis of Phosphonodisaccharide Analogue from *Neisseria Meningitidis a* capsular polysaccharide** **e-mail: cristina.santi@unimi.it**	**PC-8**

Stefano D'Errico, Giorgia Oliviero, Nicola Borbone, Bruno Catalanotti, Valeria Costantino, Gennaro Piccialli, **Maria Marzano** (*Università di Napoli*) **"Northern" Ribose and Pyrophosphate Modified cADPR Analogues: a Novel Class of Potential Ca^{2+} Mobilizers** **e-mail:** maria.marzano@unina.it	**PC-9**
M. Arcuri, **R. Di Benedetto**, A. Cunningham, A. Saul, C.A. MacLennanc, F. Micoli. (*GSK Vaccines S.r.l. Siena*) **The Influence of Conjugation Variables on the Ddesign and Immunogenicity of a Glycoconjugate Vaccine against *Salmonella* Typhi** **e-mail:** roberta.x.di-benedetto@gsk.com	**PC-10**
Matteo Gaglianone, **Maria Elena Laugieri**, Andrea Benzi, Michela Tonetti (*Università di Genova*) **The 6-deoxy hexoses metabolism in trichomonas vaginalis** **e-mail:** melena.laugieri@gmail.com	**PC-11**
Rosa Ester Forgione, Roberta Marchetti, Alba Silipo, Antonio Molinaro (*Università di Napoli*) **Study of the interaction between sialic acid-binding immunoglobulin- Type Lectins (SIGLEC) and sialylated glycans for the development of a new generation of immunomodulators** **e-mail:** rosaester.forgione@unina.it	**PC-12**
Rosa Ester Forgione, Cristina Di Carluccio, Roberta Marchetti, Antonio Molinaro, Alba Silipo (*Università di Napoli*) **The interplay between NMR spectroscopy and molecular modeling: a powerful strategy to investigate protein-glycoconjugate interactions** **e-mail:** rosaester.forgione@unina.it	**PC-13**
Cristina Di Carluccio, Roberta Marchetti, Alba Silipo and Antonio Molinaro (*Università di Napoli*) **NMR study of interactions between siglecs and synthesized complex-type n-glycans: the case of SIGLEC-2** **e-mail:** cristi.dicarluccio@gmail.com	**PC-14**
Linda Rabbachin, Federica Mazzieri, Laura Russo, Marta Pozzi, Eszter Prépost, Krisztina Kerekes, Zsuzsanna Csikos, **Francesco Nicotra** (*Università di Milano-Bicocca*) **Functionalization of Chitosan-PGA based nanoparticles for Multimodal diagnosis in type I diabetes regenerative therapy** **e-mail:** Francesco.nicotra@unimib.it	**PC-15**
Oscar Francesconi, Marco Martinucci, Lorenzo Badii, Cristina Nativi, Stefano Roelens (*Università di Firenze*) **Selective Fucose Recognition in water by a biomimetic synthetic receptor.** **e-mail:** oscar.francesconi@unifi.it	**PC-16**

Alessio Corrado, Francesco Berti, Luigi Panza, Roberto Adamo (*GSK Vaccine, Siena*) **A new set of orthogonal protecting groups for a disaccharide synthon leading to conjugable Lipid A analogues** **e-mail: alessio.x.corrado@gsk.com**	**PC-17**
Francesca Nonne, Daniela Proietti, Riccardo De Ricco, Luigi Lay, Maria R. Romano, Roberto Adamo (*GSK Vaccine, Siena*) **Identification and synthesis of analytical standards for quantification of N. Meningitidis capsular polysaccharide serogroup A carba analogues** **e-mail: francesca.x.nonne@gsk.com**	**PC-18**
Felicia D'Andrea, Lorenzo Guazzelli, **Camilla Baldini**, Giorgio Catelani (*Università di Pisa*) **Synthesis of an aminoacid containing analogue of the *streptococcus pneumoniae* type 14 repeating unit** **e-mail: felicia.dandrea@unipi.it**	**PC-19**
Francesca Gado, Marco Macchia, Clementina Manera, Felicia D'Andrea, Lorenzo Guazzelli, **Lorenzo Silicani** (*Università di Pisa*). **Synthesis of glyco-conjugates of 1,2-dihydro-2-oxo-pyridine-3-carboxamides as new modulators of endocannabinoid system** **e-mail: francesca.gado@for.unipi.it**	**PC-20**
Felicia D'Andrea, **Elisabetta Parodi,** Sebastiano Di Pietro, **Valeria Di Bussolo** (*Università di Pisa*) **Stereoselective synthesis of new imino analogues of minimal epitope Manα(1,2)Man as potential DC-SIGN ligands** **e-mail: valeria.dibussolo@unipi.it**	**PC-21**
Stefano Giuntini, Evita Balducci, Linda Cerofolini, Enrico Ravera, Marco Fragai, Francesco Berti, Claudio Luchinat (*Università di Firenze*) **Characterization of glycosylated e. coli L-asparaginase II by NMR** **e-mail: giuntini@cerm.unifi.it**	**PC-22**
Marco Fragai, Stefano Giuntini, Linda Cerofolini, Enrico Ravera, Claudio Luchinat (*Università di Firenze*) **Atomic structural details of a protein grafted onto gold nanoparticles** **e-mail: fragai@cerm.unifi.it**	**PC-23**

Biao Yu received a Bachelors of Science in Nuclear Chemistry from Peking University in 1989. He went on to obtain a PhD degree in Organic Chemistry from the Shanghai Institute of Organic Chemistry (SIOC). In 1995, Dr. Yu moved to New York University to complete one year of postdoctoral training and was then hired as an Assistant Professor and then Professor at the SIOC. Professor Yu's research efforts have been focusing on chemical synthesis of complex glycoconjugates and oligosaccharides (ex. mammalian glycosaminoglcans and microbial antibiotics) as well as diverse glycosides from plant and marine species. He has authored over 250 papers and 20 patents. He is currently the Director of the State Key Laboratory of Bioorganic and Natural Products Chemistry and, since 2012, Deputy Director of SIOC.



Claudia Bello received her Master degree in chemistry with honours in 2003 at the University of Basilicata, Italy. After a one year specialization course at the University of Pavia, which included an internship at a Swiss pharmaceutical company in Aarau (CH), she moved to Switzerland to join the group of Prof P. Vogel at the Ecole Polytechnique Fédérale de Lausanne (EPFL), where she obtained her PhD in chemistry and chemical engineering in 2009. She received then an Innogrant from the VPIV of the EPFL to continue her research during one year of postdoc. In 2010 she moved to Munich (Germany), as she was awarded an A. von Humboldt foundation fellowship for postdoctoral researchers, joining the group of Prof C. F. W. Becker at TUM (her second host was Dr A.-C. Gavin at the EMBL-Heidelberg). Subsequently, she obtained a six years position as Senior Researcher at the University of Vienna (Austria). She is an awardee of the Montalcini Program for young researchers, call 2014, and is currently Assistant Professor (RTD-b) at the Department of Chemistry of the University of Florence.

Prof. Alba Silipo, Professor of Organic Chemistry

1996-2001: Degree in Chemistry at University of Naples Federico II, mark 110/110 summa cum laude..

September-December 2001: Guest at the Research Center Borstel, Germany, in the laboratory of Structural Biochemistry, directed by Prof. Otto Holst.

January-June 2004: Guest at the Institute of Chemistry, University of Luebeck, Germany, in the laboratory directed by Prof. Thomas Peters.

December, 14th 2004: Awarding of PhD in Chemistry at the University of Naples Federico II.

2005-2006 One year postdoctoral EU Marie Curie Training Fellow within the SACC-SIG-NET project, supervision of Prof. Jesús Jiménez-Barbero, CSIC, Madrid.

2006-2007 One year post-doctoral grant (Work on the Job e Alta Formazione).

September-November 2007, March 2008 Contracts supported by Prof. Hartmut Oschkinat, Leibniz-Institut für Molekulare Pharmakologie, Berlin, on the study of lipopolysaccharide micelles and liposomes via SS-NMR.

November 2007 Researcher in Organic Chemistry, University of Naples Federico II.

November 2015: Associate Professor in Organic Chemistry, Department of Chemical Sciences, University Federico II.

Italian National Scientific Qualification: eligible for a full professorship in Organic Chemistry (03/C1 Organic Chemistry) (2014-2020)

Teaching: Undergraduate and post-graduate levels (2 Master programs), supervisor of bachelors and Masters projects. Five PhD thesis supervised; Currently supervising four PhD students (1 Italian, 2 French and 1 Polish), two postdocs

2015- Organic Chemistry course and Organic Analysis course, University of Napoli Federico II.

Member of the Editorial Board of Marine Drugs, http://www.mdpi.com/journal/marinedrugs/editors

Dr. Silipo is co-author of 120 publications on international journals and 7 publications on books

PI of the following projects:

1. H2020-MSCA-ITN-2014, Proposal number: 642157, " TOLLerant", PI as Beneficiary partner
2. PNRA16_00089, Linea A1, 2017-2019

3. EU COST Action BM1003 2011-2014), Vice MC
4. Progetto Galileo 2014-2015 - Project number: G14-23 (France project code: 32238SL), PI
5. Progetto Canaletto, 2013-2015, PI
6. FFC#11/2012 2012-2013
7. FFC#16/2009 2009-2011

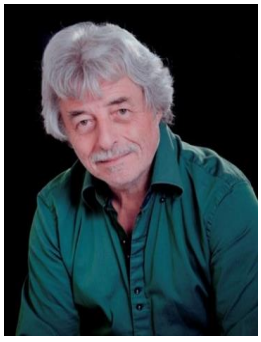
Awards

- III European prize of the RSEQ CHEMICAL BIOLOGY DIVISION (GEQB) to young group LEADERS", 2017 that acknowledges and recognises outstanding young research group leader that contribute to the field of Chemical Biology. (presented at 16th Iberian Peptide Meeting (16EPI) / 4th Chemical Biology Group Meeting (4GEQB), https://eventum.upf.edu/10279/detail/16th-iberian-peptide-meeting-and-4th-chembio-group-meeting-barcelona.html)
- IEIIS Nowotny Award, 2016, presented at the 14th Biennial Meeting of the International Endotoxin and Innate Immunity Society in Hamburg in September 2016 (http://www.ieiis.org/Nowotny),
- Medaglia Giacomo Ciamician from SCI (Italian Chemical Society) 2012,) (34° Convegno Nazionale della Divisione di Chimica Organica – Pavia 10-14/9/2012); http://www.soc.chim.it/it/node/566)
- VII edition of "Giacomino Randazzo" PhD award in 2004.

Manuel A. Coimbra (mac@ua.pt)

- ✓ Associate Professor at the Department of Chemistry of University of Aveiro, Portugal. BSc in Biochemistry (University of Porto, 1985) and PhD in Food Biochemistry (University of Aveiro, 1993). Habilitation in Chemistry ((University of Aveiro, 2003).
- ✓ Professor of Biochemistry and Food Chemistry, and Polysaccharides Chemistry. Supervisor of 6 Post-Doc students, 18 PhD students with completed thesis, and 69 MSc students.
- ✓ Principal research interests: Food chemistry and biochemistry, carbohydrate chemistry, polysaccharides chemistry; volatile compounds; phenolic compounds.
- ✓ Regular collaboration with food industry.
- ✓ Editor of Carbohydrate Polymers (Elsevier, IF2016=4.811).
- ✓ President of Additives and Food Chain Contaminants Panel in the aim of the Scientific Council of Portuguese Economic and Food Safety Authority (ASAE).
- ✓ Authorship of 4 Patents, 208 Papers (h=40), 3 Books, and 22 Book Chapters**.**

Serge Perez (CNRS, Grenoble) was born in Perigueux, France, and graduated, in crystallography, from the University of Bordeaux, France. He then spent one year at the Institute of Molecular Biology at the University of Oregon, Eugene, USA and subsequently three years at the Department of Chemistry at the University of Montreal, Canada. He returned to France upon accepting a permanent position as a Junior Scientist of the Centre National de le Recherche Scientifique (CNRS) at Centre de Recherches sur les Macromolecules Végétales (CERMAV), Grenoble, and he spent a sabbatical year working for Eastman Kodak in Rochester, USA. Later on, he moved on to Nantes, France, at the Institut de la Recherche Agronomique, where he was awarded a position as Senior Research Scientist to start a research group in Molecular Engineering. In January 1996 he moved back to Grenoble to become Chairman of the CERMAV, and hold this position for 11 years. He left this position to the European Synchrotron Radiation Facility to take up the position of Scientific Director in charge of the Life Sciences, Chemistry and X-ray Imaging programs. After completing his term, he returned to the CERMAV in 2012. Since January 2014 he is a staff member of the Department of Molecular Pharmacochemistry, a joint research unit between CNRS and University Joseph Fouirer in Grenoble.



His research interests span across the whole area of structural and conformational analysis of oligosaccharides, polysaccharides, glycoconjugates and protein–carbohydrate interactions in solution and in the solid state. This includes interests in computational chemistry and molecular modeling, crystallography, NMR spectroscopy, along with the structure-function and structure-properties relationship. He is the author of more than 280 research articles, review articles and book chapters in this area, which gave rise to about 11000 citations. Beside his broad range interest in the field of structural glycoscience, Serge Perez is involved international and international committees related to the evaluation of scientific research and to

the structuring of multilateral collaborative schemes. He is also involved in management duties at the doctoral levels and in the creation of glyco-biotechnology companies. He is also the founder of the internet site: glycopedia.eu, an initiative which addresses aiming at promoting the field of glycoscience throughout the publication of educational e-chapters and the publication of e-news.

Fabrizio Chiodo

Fabrizio studied at the University of Palermo (Italy) where he got his Master degree in Pharmaceutical Sciences in 2008. At the end of 2008 he stared his PhD in the group of S. Penades (CIC BiomaGUNE, San Sebastian, Spain). In 2011 Fabrizio moved to the Molecular Cell Biology Immunology Department at the VU Medical Center in Amsterdam (The Netherlands). He worked simultaneously in the group of Y. van Kooyk and I. van Die. In March 2013 Fabrizio defended his PhD in Applied Chemistry with a Thesis entitled : Gold Glyconanoparticles as Multivalent Nanocarriers for Carbohydrate-Antigens. He then moved back to the group of Y. van Kooyk as guest Postdoctoral Researcher. In November 2013 Fabrizio moved to Leiden (The Netherlands) in the group of C.H. Hokke working on a project that involved goldnanoparticles coated with Schistosoma mansoni carbohydrate-antigens. In 2014 Fabrizio was granted with a Veni fellow from NWO. From November 2014 he is a senior Postdoctoral Researcher working in the group of J. Codee at the Bio-Organic Synthesis Department. His projects are mainly related with the role of carbohydrates in modulating adaptive and innate immune responses exploring goldnanoparticles as a chemical tool to investigate these responses both in vitro and in vivo. From 2013 Fabrizio started a strong collaboration with V. Verez-Bencomo (Havana, Cuba) to study the role of bacterial polysaccharides in modulating innate responses. From March 2016 Fabrizio is Invited Lecturer at the Faculty of Chemistry at Havana University. He recently got a grant from the Amsterdam Infection and Immunity Institute to follow his research on carbohydrate-based vaccines.

Dr. Christoph Rademacher earned his BSc in Molecular Biotechnology (2004) and MSc in Molecular Life

Science (2006) at the University of Lübeck. In 2009, Dr. Rademacher received his doctorate from the same University, where he performed studies under the supervision of Prof. Dr. Thomas Peters in the Department of Chemistry working on virus/carbohydrate interactions using NMR spectroscopy. During these years, he also worked in Prof. Dr. David R. Bundle's and Prof. Dr. Todd Lowary's laboratories at the Alberta Ingenuity Center for Carbohydrate Science in Edmonton (Canada) and in Dr. Daron Freedberg's group at CBER/FDA in Bethesda (USA). He then underwent postdoctoral training with Prof. Dr. James C. Paulson at The Scripps Research Institute (USA) in the Department of Chemical Physiology, where he entered the field of glycoimmunology. Since December 2011, Dr. Rademacher is appointed at the Max Planck Institute of Colloids and Interfaces in the Department of Biomolecular Systems, where he became Emmy-Noether Research Group Leader in June 2012. Since 2017, Dr. Rademacher holds an ERC Starting Grant. His research is focused on the development and application of novel molecular probes to understand the role of carbohydrates in immune cell regulation.

Bertini Sabrina. Date and place of birth: March 25th , 1971 Milan (Italy).

Education: 1996: Graduation in Chemistry, at University of Milan, dissertation on “Sintesi e caratterizzazione di nuovi materiali a base di ciclodestrina” Supervisor: Prof. F. Nicotra. 1999: Specialization in Science of Polymers “G. Natta”, Department of Macromolecules, Polytechnic of Milan, dissertation on “Sintesi e caratterizzazione di materiali polimerici a base di ciclodestrina” Supervisor: Prof. P. Sozzani.
Experience: 1996-1999 – Research fellow at the at the G. Ronzoni Insitute, Milan, Italy. 1999-2002 Reserchер at G. Ronzoni Insitute. From 2002 to present head of the Physical Chemistry Unit of the Carbohydrate Sciences Group of the G. Ronzoni Institute.
Activity: I am a senior researcher at the Institute of Chemical and Biochemical Research "Giuliana Ronzoni, and head of the physical chemistry unit; I contributed with original research in the field of polysaccharide characterization with special emphasis on the development of methods for the determination of their chemical and physical properties both in solution and in solid. I work with polysaccharides of different origins such as starch, cellulose, chitosan and glycosaminoglycans. I have a good experience of participating

in interdisciplinary research projects both nationally and internationally (EU, NIH), as documented by numerous publications. In particular, 30 published paper, 1 patent and several communications at national and international symposium.
Other information: In 2000 Awards of the Medal for best young researcher, Carbohydrate Group of Italian Chemical Society. I was teacher at Bruker user meeting about NMR solid state and at Malvern user meeting about molecular weight determination of polymers.

Laura Russo's research experience dates to 2009, as PhD student in the BioOrganic research group of

University of Milano-Bicocca, working on a multidisciplinary project exploiting glycoscience in the field of nano- and bio-materials for tissue engineering. In 2010 Dr. Laura Russo joined the research group of Prof. Julian R. Jones, at Imperial College of London, as Visiting Researcher, working on the design and synthesis of hybrid biomaterials for tissue regeneration. From 2013 to 2015 she worked, as Post Doc Fellow, at University of Milano, where she was unit coordinator of a research project founded by CARIPLO Foundation on the design and synthesis of smart biomaterials through the chemical derivatization with carbohydrates as signal biomolecules. In 2016 Dr. Russo awarded a SFI Starting Investigator Research Grant (SIRG) to conduct her research on Glycofunctionalized hydrogels and biomaterials for tissue engineering applications. From 2017 Dr Russo is Assistant Professor at University of Milano-Bicocca, and Visiting Researcher at Cùram– NUIG, Ireland**.** Since 2009 Dr. Russo is co-author of 43 publications on peer-reviewed international scientific journals with impact factor, (15 as first author, 1 as corresponding author, 5.8 publications/year) 2 Patents, 9 book chapter on biomaterial functionalisation and carbohydrate chemistry, more than 60 poster communications, 2 oral communications and 5 invited lectures, at national and international meetings and several press reviews.

Cinzia Colombo

After taking the scientific high school diploma in 2003, I attended my graduate studies in Chemistry at the Università degli Studi di Milano (UNIMI), from 2003-2008. My master thesis, entitled "Synthesis of pseudo disaccharides as inhibitors of DC-SIGN" involved the synthesis of a small library of pseudo-di-mannoside derivatives with the purpose of blocking the lectin DC-SIGN.
I remained at the Università degli Studi di Milano to undertake my PhD studies in Chemical Science with specialization in organic chemistry with Prof. Anna Bernardi, from 2009-2012. The thesis was entitled "Design and synthesis of unnatural glycopeptides" and the project dealt with the synthesis of α-N-linked glycopeptides, unnatural molecules which may introduce structural diversity by mimicking and/or interfering with molecular recognition events.
After my PhD studies, I took up a postdoctoral fellowship at CNR-ISTM (Milan) and I worked on the synthesis of peptidomimetics as cadherin's inhibitors for the FIRB project 'Futuro e Ricerca' coordinated by Dr. M. Civera (UNIMI). I moved to France in November 2012 to join Dr. I. Huc group, where I worked as a postdoctoral fellow for one year at the Institut Européen de Chimie et Biologie. The research project was part of the European FP7-People IAPP project FOLDAPPI and dealt with the synthesis and development of helical aromatic amide foldamers to inhibit protein-protein interactions.
In January 2014 I moved to Simon Fraser University, Canada, where I started a Marie Curie International Outgoing Fellowship (IOF-FP7-People) on the "Design, synthesis and evaluation of potential group-1 and group-2 neuraminidase inhibitors". This project was continued at the Università degli Studi di Milano, as part of the IOF return phase.
I am currently working as a postdoctoral Fellow at Università degli Studi di Milano (UNIMI) under the supervision of Prof. L. Lay. My main project is focused on the synthesis and immunological evaluation of Salmonella Typhi carbohydrate antigens.

GLYCOCONJUGATE VACCINES: LESSONS LEARNED AND NEW TREND

Paolo Costantino

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Glycoconjugate vaccines are composed of a carbohydrate moiety covalently linked to a protein carrier. Polysaccharides are T-cell independent antigens able to directly stimulate B cells to produce antibodies. Disease burden caused by polysaccharide-encapsulated bacteria is highest in the first year of life, where plain polysaccharides are not generally immunogenic limiting their use as vaccines. This limitation has been overcome by covalent coupling carbohydrate antigens to proteins that provide T cell epitopes and since the eighties glycoconjugate vaccines have been introduced in the arsenal of available weapons for prevention of infectious diseases.

Although licensed glycoconjugate vaccines are produced by conjugation of capsular polysaccharides or their fragments, other classes of bacterial surface carbohydrates represent attractive targets for developing vaccines against pathogenic bacteria.

There are different approaches to produce glycoconjugate vaccines. Progress in carbohydrate chemistry is making feasible the developing of synthetic glycoconjugate vaccines. In some cases chemo-enzymatic approaches allow the enzymatic elongation in vitro of bacterial oligosaccharides ready for conjugation to carrier proteins. Strategies based on the expression of glycoconjugates in engineered E.coli or on non-covalent association of polysaccharide to proteins are emerging.

Many elements may impact the product profile of a conjugate vaccine: conjugation chemistry, length of the saccharide moiety, density of glycosylation, O-acetylation of the carbohydrate moiety, stability and carrier protein, the latter being exploited recently with the additional role of protective antigen. This lecture will give and overview on glycoconjugate vaccines including preparation approaches and key variables impacting their product profile.

CHEMICAL SYNTHESIS OF MARINE SAPONINS

Biao Yu

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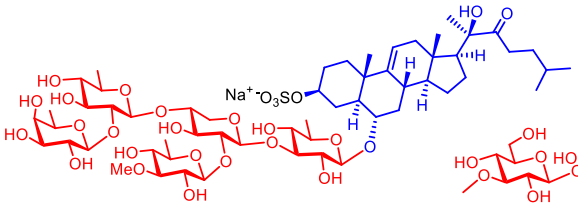
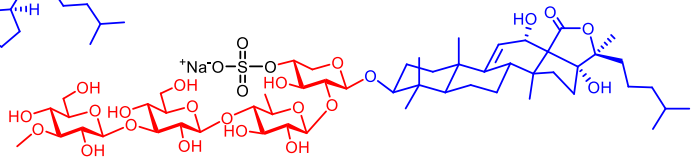
Marine saponins are characteristic metabolites of the slow-moving starfish and sea cucumbers. These complex glycosides of steroids or triterpenes are believed to be the defense chemicals against parasites and predators, therefore are expected to exhibit a wide range of biological and pharmacological properties. Isolation of sufficient amounts of homogeneous marine saponins from the natural sources is always a formidable task, such hampering in depth studies on their activities. Herein we report our recent efforts on the chemical synthesis of these complex natural products, specifically, the successful synthesis of asterosaponins Goniopectenoside B and Astrosterioside A, polyhydroxysteroid starfish saponins Linckosides A and B, and sea cucumber saponin Echinoside A.



Goniopectenoside B
Chem. Eur. J. 2013



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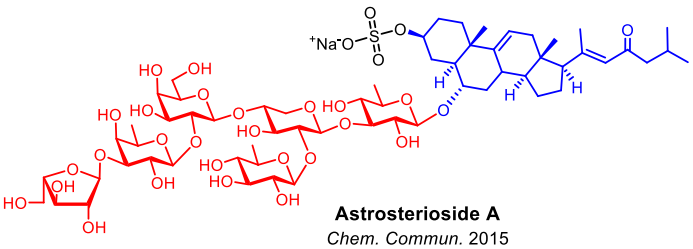
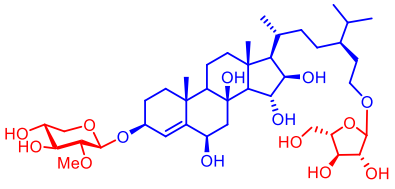
Angew. Chem. 2017



Astrosterioside A
Chem. Commun. 2015



Linckoside A
J. Am. Chem. Soc. 2015

AN AUXILIARY-MEDIATED APPROACH FOR THE SIMPLE PREPARATION OF COMPLEX GLYCOPEPTIDES AND GLYCOPROTEINS

Claudia Bello

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Having access to homogeneous proteins carrying complex posttranslational modifications (PTMs) is essential for studying the role of the PTMs in protein function and misfunction.
We established a strategy for the synthesis of peptides carrying multiple and complex PTMs, focusing at first on the preparation of homogenously glycosylated peptides. The tumor marker MUC1, a protein abundantly *O*-glycosylated in his extracellular domain, was chosen as synthesis target. Chemically synthesized mucin peptides are conjugated to a photocleavable ligation auxiliary [1], obtained via multistep synthesis, that supports native chemical ligation (NCL) and carries a PEG polymer. This facilitates effective enzymatic glycosylation and recovery of the resulting glycopeptides without the need for chromatographic steps [2]. It also gives access to glycosylated peptide α-thioesters that are otherwise inaccessible [3]. The conjugates are combined to each other via auxiliary-mediated NCL and the ligated products are recovered as non-protected glycopeptides after UV irradiation.

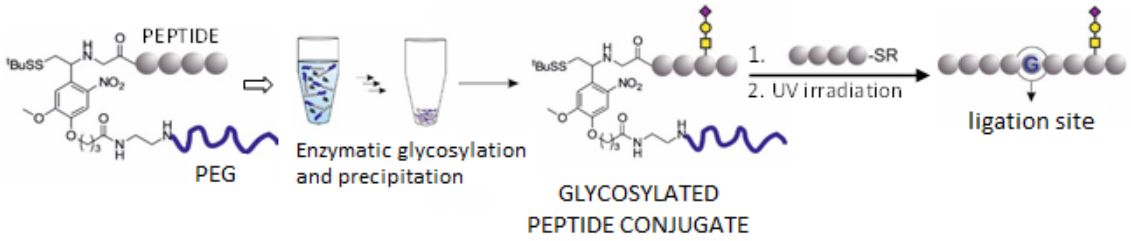

Figure 1 *The PEGylated auxiliary supports sequential glycosylation and easy recovery of glycopeptides and mediates NCL. The native glycopeptide is obtained after photocleavage.*

I am building a library of mucin polypeptides with multiple glycosylations that will be used in proteomic studies to provide new insights into the role of glycosylation in mucin functions and cancer progression.
I am expanding the approach to sequential ligation [4] and to a combination of sequential glycosylation and sequential ligation, to gain access to complex homogenous glycoproteins.
Moreover, we are extending the method to ligation at sites different from glycine and to other PTMs, in order to broaden it toward the synthesis of any kind of homogeneously posttranslationally modified protein.

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POLYSACCHARIDES CHEMISTRY AND FOOD APPLICATIONS: FROM SULPHUR DIOXIDE ALTERNATIVES TO ACRYLAMIDE MITIGATION

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Food polysaccharides have applications that are defined by their nutritional and structural characteristics: the energy providers, based on (α1→4)-Glc residues, such as starch polysaccharides, and the "non-starch polysaccharides", including the polysaccharides not mobilized by the human digestive enzymes, which act as dietary fibre and contribute to the modulation of the rheological and textural characteristics of the foods. Polysaccharides can also be used as materials components of biobased food packaging. Chitosan, a polysaccharide with animal and fungi origin, composed by (β1→4)-GlcN residues, and pectic polysaccharides, derived from plant cell walls, composed by (α1→4)-GalA repeating units, are examples of polysaccharides with a vast number of applications in food.

Chitosan, due to its nontoxicity, biocompatibility, and antimicrobial properties, is able to form edible films. Therefore, chitosan becomes a suitable candidate for a wide range of beverage applications including clarification, preservation, encapsulation, and packaging [1]. However, for food applications, the use of chitosan in the form of films has been limited due to their high degradability in aqueous acidic media and low resistance. To overcome this limitation, the cross-linking of chitosan with genipin, a natural and non-cytotoxic compound extracted from gardenia fruit, has been proposed, enhancing the films mechanical strength and chemical stability, rendering them practically insoluble in acidic aqueous solutions [2]. This allows to exploit chitosan antimicrobial and antioxidant properties for wine preservation as an alternative to the use of sulphur dioxide [3].

Polymeric sugar acid structures, like those present in pectic polysaccharides, having a high ratio of carboxylic groups in relation to the reducing end, has been shown to provide the required medium acidity to minimize the formation of acrylamide in cooked foods. Although relevant for all diets, this property is even more important for fructose-rich foods, such as the biscuits eaten by diabetics, where the content of acrylamide formed is usually high. As the main route for acrylamide formation is the reaction between reducing sugars and asparagine, this reaction is minimized at low pH. These conditions can be provided by deesterified and purified pectic polysaccharides without contributing to the sour taste usually associated with acid foods [4].

Acknowledgements: Thanks are due to FCT/MEC for the financial support of QOPNA (FCT UID/QUI/00062/2013) through national funds, co-financed by the FEDER, within the PT2020 Partnership Agreement.

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FRAGMENT-BASED DESIGN OF CARBOHYDRATE RECEPTOR LIGANDS

Christoph Rademacher

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Mammalian carbohydrate receptors determine many aspects of life such as cell migration, pathogen recognition, uptake and processing, as well as cellular communication in multicellular organisms. Hence, it is not surprising to find many carbohydrate receptors on cells of the innate immune system, utilizing these proteins to differentiate self from non-self and shape an appropriate immune response. Their unique expression patterns on defined immune cell subsets allow to specifically target these cells in their complex biological environment. Consequently, carbohydrate receptors of the innate immune system as initiators and regulators of an immune response, are attractive targets for the therapeutic modulation.

However, small molecule inhibitors of these carbohydrate-protein interactions are sparse. Carbohydrate-binding sites are often rather featureless and flat and are therefore considered challenging [1]. Thus far, attempts to apply high throughput screening have shown limited success. Our approach follows fragment-based design principles, in which fragments of drug-like molecules are screened using sensitive biophysical techniques such as 19F NMR, STD NMR, HSQC NMR, and SPR [2-4]. Additionally, computer-based approaches as well as screening on chemical fragment microarrays and a recently developed cell-based screening assay complement these insights. The chemical probes we have developed are applied to basic questions in immunology and small molecule targeted delivery to specific immune cell subsets [5].

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CHEMICAL PHYSICAL CHARACTERIZATION OF POLISACCHARIDES AND THEIR INTERACTION WITH PROTEINS

Sabrina Bertini

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Polysaccharides are biopolymers commonly found in living organism and are known to reveal the physiological functions by performing a specific conformation function of chemical structural parameters, such molecular weight distribution, branching or liner structure, charge distribution etc. Therefore, structural analysis may offer the most fundamental knowledge to understand the functions of polysaccharides, but the diversity and irregularity of polysaccharide chain make it a formidable task.

Glycosaminoglycans (GAGs) are complex polysaccharides consisting of uronic acid and hexamine repeating disaccharide units with different length and variable sequences containing sulfated or non-sulfated groups. Sulfated glycosaminoglycans like heparin, heparan sulfate and dermatan sulfate have important biological activities, associated with interaction with several proteins, including chemokines and growth factors, to regulate both physiological and pathological processes.

Otherwise, Hyaluronic acid (HA) is a non-sulfated GAG, and is found throughout the body, from the vitreous of the eye to the extracellular matrix of cartilage tissues. HA can be modified in many ways to alter the properties of the resulting materials, including modifications leading to hydrophobicity and biological activity, and these materials have been clinically used as medical products for over three decades. More recently, HA has become recognized as an important building block for the creation of new biomaterials with utility in tissue engineering and regenerative medicine. The application of HA derivates is related to the viscosity of polysaccharide in solution and the molecular weight distribution. Since polysaccharide structure is very important for the bioactivity, several qualitative and quantitative methods have been developed for the characterization of GAGs, in e.g. Nuclear Magnetic Resonance (NMR) for the determination of structural, dynamical, conformational and intermolecular binding aspects of polysaccharides, Mass Spectroscopy for the detection of intact ions in mixtures of unknown compounds, High Performance Gel Permeation Chromatography, for the determination of the molecular weight of GAGs, Photo Correlation Spectroscopy for the analysis of GAGs size and Calorimetry techniques for the study of the interaction between GAGs and protein and for the study of thermodynamic parameters.

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GLYCANS AND BIOMATERIALS: FROM NANOMEDICINE APPLICATIONS TO 3D BIOPRINTED CELL CULTURE MODELS

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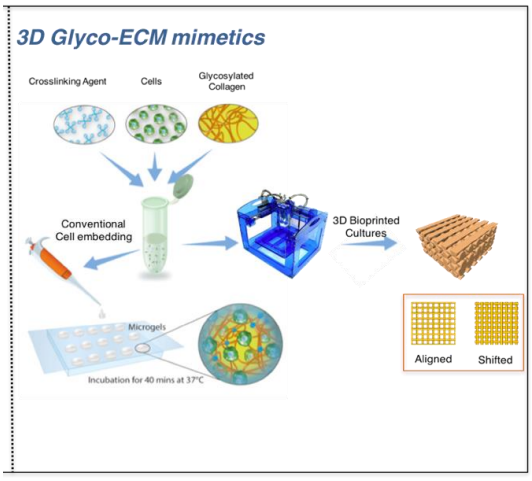
Glycans represent a largely untapped resource for biological discovery as well as unanticipated therapeutic and diagnostic opportunities. They are ubiquitous in all living cells and organisms, where they serve essential functions ranging from acting as structural components to regulate physiological and pathological processes [1]. The glycome is also a highly dynamic molecular entity that mirrors a biological milieu and confer to cell microenvironment an important regulatory role. The design of new synthetic strategies aimed to obtain new glycosylated nano- and 3D bioprintable materials biologically inspired, has high impact in different biomedical fields, from nanomedicine, to tissue engineering and cell biology studies [2,3]. Taking inspiration from glycans role in cell-cell and cell-extracellular matrix interactions, different functionalized glyco-tools are needed. Here in this talk functionalization strategies of different materials and their biomedical applications will be presented.



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STRATEGIES FOR THE SYNTHESIS OF URONIC ACID OLIGOSACCHARIDES. *SALMONELLA TYPHI* VI ANTIGEN AS A CASE STUDY

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Uronic acids belong to an important group of carbohydrates and are defined as monosaccharides in which the primary alcohol is oxidized to a carboxylic acid.1 Examples of polysaccharides containing uronic acids are alginate (D-mannuronic and L-guluronic acids),2 pectin (D-galacturonic acid),3 glycosaminoglycans like heparin (D-glucuronic and L-iduronic acids).4 The abundance of uronic acids in nature and their application in food industry and medicine have stimulated the development of methodologies for their preparation.5 The synthesis of aldohexuronic acid glycosides is particularly challenging due to the electronwithdrawing carboxylic group at C5 that decreases the sugar reactivity in glycosylation reaction. In the framework of this lecture, the general strategies for the synthesis of uronic oligomers ("pre-glycosylation oxidation" and "post-glycosylation oxidation" approaches) will be discussed, with a particular focus on galacturonic acid oligomers. The example of Salmonella Typhi capsular polysaccharides,6 an anionic polymer composed by α-(1→4)-linked N-acetyl galactosaminuronic acid repeating units, will be taken as a case study and described in detail.

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UNDERSTANDING THE ROLE OF GLYCOCONJUGATES AS KEY MOLECULES FOR CELL SURVIVAL AND COMMUNICATION IN PROKARYOTES AND EUKARYOTES

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Carbohydrates are rarely simple molecules; they occur either alone or covalently linked to proteins and lipids (glycoconjugates). This ubiquitous occurrence translates in many roles and functions such as structurally rigid and plastic elements, energy storage in living cells, interactions between viruses, bacteria and the surfaces of mammalian cells and molecular recognition for intracellular trafficking.

Innate immunity is the first line of defence against invading microorganisms in vertebrates and the only line of defence in invertebrates and plants and therefore plays a crucial role in the early recognition and subsequent triggering of a pro-inflammatory response to invading pathogens. This mechanism relies on recognition of evolutionarily conserved structures on microbes, termed microbe-associated molecular patterns (MAMPs), through a limited number of germ line-encoded pattern recognition receptors. MAMPs are characterized by being invariant among entire classes of microbes, essential for their survival, and distinguishable from "self". Microbial glycoconjugates such as lipopolysaccharide and peptidoglycan act as MAMPs in eukaryotic/bacteria interactions. Besides their general architectural principle, a number of subtle chemical variations are at the basis of the dynamic host-guest recognition that in case of pathogens is followed by the innate response and in case of symbiosis is followed by its suppression. Therefore, the structural study of such glycoconjugates involved as virulence or beneficial factors in animal or plant interactions is a pivotal pre-requisite for the comprehension at molecular level of the innate immune mechanisms.

Likewise, sialic acid binding immunoglobulin (Ig)-like lectins (Siglecs) bind sialic acids which are the most prevalent residues located at the terminal position of (N- or O-) glycan structures on cell surface glycoproteins and glycolipids found on all mammalian cells. Given their capability to recognize a common structural element of the mammalian glycome, the Siglecs are nowadays recognized for their role in helping immune cells to distinguish between “self” and “non-self”. In contrast to others immune cell proteins, which recognize MAMPs and damaged cells (DAMPs), indeed, the Siglecs detect peculiar ligands that are determinants of “self”, referred to as self-associated molecular patterns (SAMPs). The Siglecs influence almost every cell in the immune system and modulate a plethora of immune responses which are of relevance both in health and disease,

In this communication, I will show some examples of isolation, structure determination of complex glycoconjugates and their contribute to the cell survival as well as their interaction with their receptor(s).

A COMPUTER TOOL BOX FOR GLYCOSCIENCE

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Information searching and data extraction are benefitting from the formidable contribution of computational science and technology. Glycoscience is now making use of the development and implementation of robust and validated informatics toolbox, which enables accurate and fast determination of complex carbohydrate sequences extendable to 3D prediction, computational modeling, data mining and profiling. The course will present the scope of applications that are available to be used by everyone in its current research practice.

DESIGN AND PREPARATION OF MULTIVALENT GOLD NANOPARTICLES TO INTERROGATE AND INVESTIGATE IMMUNOLOGICAL PROPERTIES OF CARBOHYDRATE

Fabrizio Chiodo

Leiden Institute of Chemistry, Bio-Organic Synthesis Department and Leiden University Medical Center, Parasitology Department

Different questions are still open in the field of carbohydrates-immunology: what is the mechanism of action of conjugated-vaccines? How can we improve antibody titers and quality? Can we design better vaccines decreasing doses and recall injections?

In trying to answer some of these questions, chemistry and its multivalent/multifunctional tools can play a key role.

During this didactic lecture, the design and preparation of carbohydrate-coated gold nanoparticles will be described as tool to interrogate and investigate different aspects of carbohydrates-immunology. For example, exploring the proper design of carbohydrate-coated gold nanoparticles, we proved that some non-mammalian monosaccharides (like Galactofuranose) are able to trigger pro-inflammatory responses on human antigen presenting cells especially when clustered on the gold nanoparticles surface. In addition, thanks to the information and results derived from this observation, we have studied more complex bacterial polysaccharides (H. influenzae and S. pneumoniae, in collaboration with Finlay Institute in Cuba) to prove the concept of glyco-pathogen associated molecular patterns and the dual antigen/adjuvant role of some bacterial polysaccharides.

WELL-DEFINED OLIGO AND POLYSACCHARIDES AS IDEAL PROBES FOR STRUCTURAL STUDIES

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Polysaccharides are the most abundant organic materials in nature, yet correlations between their three-dimensional structure and macroscopic properties have not been established.[1] Automated glycan assembly (AGA) enables the preparation of well-defined oligo- and polysaccharides resembling natural as well as unnatural structures.[2] A collection of related compounds, modified at specific positions of the chain, is presented. These synthetic glycans are ideal probes for the fundamental study of polysaccharides, shedding light on how the modification patterns affect the polysaccharides properties (i.e. three dimensional shape and aggregation behavior). Molecular modelling simulations and NMR analysis show that different classes of polysaccharides adopt fundamentally different conformations, drastically altered by single-site substitutions.[3]

The aggregation behavior of these synthetic materials is strongly affected by the single chain conformation and the modification patterns; spherical particles as well as linear fibers are observed. The information gained through this study will guide the development of novel carbohydrate-based biomaterials with tunable properties (e.g. NPs for biomedical applications).

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SYNTHESIS AND STUDIES OF TF TUMOR-ASSOCIATED ANTIGEN MIMETIC WITH GAL-3

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Mucins are high molecular weight glycoproteins largely expressed by endothelial cells. They represent a physical protection for organs, against harmful species.1 When normal mucin became tumoral, a pathological oversimplification of the glycosidic portions occurred and saccharides, normally hidden, become exposed (identify as tumoral marker).2 Galectins are a family of proteins characterized by specific recognition domains for carbohydrates (Carbohydrate Recognition Domain, CRD)3 and with high affinities for β-galactosides epitopes. The most studied galectins are Gal-1 and Gal-3 and in particular, Gal-3 plays a fundamental role in tumoral progression which is known to be mediated by the binding with TF antigen.4 TF and Tn antigens are examples of mucin associated carbohydrate antigens ,5 highly expressed in many tumors but practically absent on healthy cells. They are characterized by an α-O-glycosidic linkage with a serine or threonine residue which belong to mucins peptidic backbone. Due to their almost exclusive presence on cancer cells, these antigens are interesting targets for the development of therapeutic cancer vaccines.6 Recently, we have developed a mimetic of Tn antigens7 and relying on previous results, we have optimized the synthesis of a TF antigen mimetic. In collaboration with the University of Lisbon were performed X-ray crystallography, NMR binding studies and isothermal calorimetry assays to unravel the molecular structural issues that govern Gal-3/TF-mimetic interaction.

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CHARACTERIZATION OF HYALURONIC ACID-TAMARIND SEEDS OFTALMIC FORMULATIONS

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Hyaluronic Acid (HA) is a non-sulfated polysaccharide belonging to glycosaminoglycans family, which underlie its application as a viscoelastic tool in ophthalmological surgery1. Tamarind seed polysaccharide (TSP) possesses properties like high viscosity, broad pH tolerance and adhesivity and this led to its application as stabilizer, thickener, gelling agent and binder in food and pharmaceutical industries2. A synergistic action between HA and TSP was wildly evaluated3,4, demonstrating the interaction between TSP and HA. In this study, the addiction of a natural small molecule (SM) to high molecular weight HA and TSP formulations, in different buffer conditions, was deeply characterized for polysaccharides/SM interaction evaluation by several techniques. High Field Nuclear Magnetic Spectroscopy was used to investigate both substances primary structures and relaxation parameters (T1 and T2) of polysaccharides in solution. The evaluation of molecular weight distribution, intrinsic viscosity and hydrodynamic radius has been done by high performance size exclusion chromatography (HP-SEC-TDA). Moreover, the hydrodynamic radius and the surface charge of the two polysaccharides (both alone and in mixture with SM) in solution were obtained directly by Photo Correlation Spectroscopy and Zeta Potential measurements and the results have been compared with HP-SEC-TDA's one. Finally, we evaluated the TSP and SM effect on hyaluronidase by HA depolymerisation kinetics. We demonstrated that the SM presence does not alter molecular weight and viscosity of both HA and TSP, but significantly reduce their size and surface charge; moreover, NMR results reveal that HA and TSP modify SM's dispersion and mobility, enhancing its bioavailability. Lastly, a deceleration effect on hyaluronidase was observed when SM and TSP are present in mixture with HA.

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FINE STRUCTURAL CHARACTERIZATION OF SULODEXIDE, A HEPARINOID DRUG

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Sulodexide is a heparinoid obtained by a patented process composed by fast-moving heparin (Fm-Hep) and dermatan sulfate (DeS) thus conferring to such a drug greater efficacy than heparin in preventing thrombus formation[1,2]. Fm-Hep is described as a 7 kDa fraction with lower anticoagulant activity than unfractionated heparin (UFH)[3] and such as UFH, it is composed by repeating disaccharide units containing an uronic acid, either iduronic (IdoA) or glucuronic (GlcA) acids, and a glucosamine which could be either N-sulfated or N-acetylated. Dermatan sulfate (DeS) is constituted by N-acetyl-galactosamine-4-sulfate linked to iduronic acid (GalNAc4S-IdoA) as the most representative but not only unit[4] and a mean molecular weight of 25 kDa is reported for DeS component of sulodexide[5]. Despite sulodexide has raised a notable interest as antithrombotic drug, only a few structural and biochemical data are available in literature and date back to 1986 when the characterization of a fraction of sulodexide (f-sulodexide) was reported[6]. By taking advantage of the combination of different techniques, such as HP-SEC/TDA, 2D-NMR and HPLC-MS, the in-depth investigation on the structural features of both the whole mixture and the isolated components was accomplished. Moreover, also the separation of fractions endowed of graded affinity towards antithrombin was achieved[7]. The strategy adopted permitted to obtain a complete profiling of sulodexide and its components offering a useful tool for possible analysis of batch production but also for the characterization of other complex drugs.

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STRUCTURE OF A PROTECTIVE EPITOPE OF GROUP B STREPTOCOCCUS TYPE III CAPSULAR POLYSACCHARIDE

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Despite substantial progress in the prevention of Group B Streptococcus (GBS) disease with the introduction of intrapartum antibiotic prophylaxis, this pathogen remains a leading cause of neonatal infection.[1] Capsular polysaccharide conjugate vaccines have been tested in Phase I/II clinical studies showing promise for further development.[2] Mapping of epitopes recognised by protective antibodies is crucial for understanding the mechanism of action of vaccines and to enable antigen design.[3] In this study, we report the structure of the epitope recognized by a monoclonal antibody with opsonophagocitic activity and representative of the protective response against type III GBS polysaccharide.[4] The structure and the atomic level interactions were determined by STD-NMR and X-ray crystallography, using oligosaccharides obtained by synthetic and depolymerization procedures. The GBS III epitope is made by six sugars. Four of the sugars are derived from two adjacent repeating units of the PSIII backbone, and two of the sugars from the branched galactose-sialic acid disaccharide contained in one of the repeating units. The sialic acid residue establishes direct binding interactions with the functional antibody. The crystal structure provides a novel insight into the molecular basis of antibody-carbohydrate interactions and confirms that the conformational epitope is not required for antigen recognition. Understanding of the structural basis of immune recognition of capsular polysaccharide epitopes can aid the design of novel glycoconjugate vaccines.

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GUT MICROBIOTA LIPOPOLYSACCHARIDES: REVERTING THE CONCEPT FROM BAD TO GOOD

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The Gut Microbiota (GM) is an essential actor in the modern concept of human health driving many host physiological and pathological processes, including immune system modulation. Accumulating evidences highlighted that studies of the immune system during health or disease cannot ignore our GM.[1] Initial sensing of microbes by the host immunity is mediated by the recognition of microbial-associated molecular patterns, such as lipopolysaccharides (LPS), which are highly conserved among bacteria, thus shared by both commensals and pathogens. The LPS structure strongly influences the biological effects on the host immune system. Defined LPS structures can act as potent agonists on the immune receptors whereas other can operate as antagonists reducing or inhibiting the cytokine production otherwise induced by toxic LPSs.[2] Thus, a crucial question to address is how the immune system distinguishes between permanently established commensals LPS and pathogens LPS. In this communication, I will show some very recently elucidated GM LPS structures and their immunological properties that revealed to express unique and interesting features. Among others, I will discuss about the structure and activity of LPS from *Bacteroides vulgatus* mpk, a commensal bacterium whose beneficial effects on health were clearly demonstrated.[3] *B. vulgatus* mpk LPS showed a lipid A as a heterogeneous mixture of mono-phosphorylated tetra- and penta-acylated species and a unique saccharide moiety. The evaluation of the immunological properties of the *B. vulgatus* mpk LPS highlighted a very weak agonistic activity on bone marrow derived dendritic cells and the capability to convert intestinal dendritic cells into a tolerant and tolerogenic phenotype mediating the maintenance of intestinal homeostasis. This effect is mainly due to the LPS structure and I clarify the phenomenon both at a molecular and biological level. Insights gained from the structural and molecular analysis of *B. vulgatus* mpk LPS and, in general, of GM LPSs might help to chemically design novel inflammation-silencing drugs as a potential alternative therapeutic approach for the treatment of inflammatory disorders.

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LIPOLYSACCHARIDES AND THE INNATE IMMUNITY: STRUCTURAL AND IMMUNOSTIMULANT STUDIES ON *Acetobacter pasteurianus* CIP103108 AND *Phaeobacter gallaeciensis* BS107 LIPOPOLYSACCHARIDE

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Lipopolysaccharides (LPS) represent one of the most important glycoconjugates found on the outer membrane (OM) of Gram-negative bacteria cell wall. These amphiphilic molecules can be divided into three chemically, biologically and biosynthetically distinct domains – a hydrophobic glycolipid portion called lipid A, anchoring the molecule in the OM, a repeating glycan termed O-polysaccharide and a core oligosaccharide connecting the two domains [1]. LPS are crucial for bacterial survival as they stabilize the membrane and protect the bacterium from negative effects of the external environment. More interestingly, they are able to trigger innate immune responses, therefore they are classed as Pathogen Associated Molecular Patterns (PAMP). The key event in the signalling is the recognition of LPS by a specific transmembrane receptor, the TLR4/MD-2 complex, leading to activation of transcription factors like NF-kB and interferon regulatory factors, which finally stimulate the production of inflammatory cytokines[2]. Here, I will present the characterization of the lipopolysaccharides extracted from two different bacteria, *Acetobacter pasteurianus* and *Phaeobacter gallaeciensis*. *Acetobacter pasteurianus* CIP103108 is an acetic acid bacterium used in production of traditional Japanese black rice vinegar *kurozu*. The beverage is believed to carry several health benefits. After isolation and purification of the cell wall components, the LPS components were separated using gel filtration chromatography. The structure of three domains was obtained using a variety of chemical and spectroscopical methods; furthermore the inflammatory and inhibitory activity of *Acetobacter* LPS was also tested. *Phaeobacter gallaeciensis* BS107 is a bacterium living in a particular ecological interaction with algae. After LPS isolation, the structure of the lipid A was revealed with use of MALDI-TOF, MS/MS and chemical methods.

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PRODUCTION OF SECOND GENERATION SUGARS FROM GIANT REED AND CARDOON AS POTENTIAL FEEDSTOCK FOR THE BIOCONVERSION INTO MICROBIAL LIPIDS

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Among lignocellulosic feedstocks, giant reed (*Arundo donax* L.) and cardoon (*Cynara cardunculus* L.) are abundant naturally growing perennial herbs of the Mediterranean region. The high biomass productivity, the annual harvesting period, the easy adaptability to different soil and climatic conditions, the ability to intensive cultivation and the appropriate chemical composition make these plants two of the most promising crops for industrial utilization in a biorefinery approach[1,2]. The chemical hydrolysis of giant reed and cardoon for the production of pentose/hexose sugars were investigated in the last years, showing an excellent reactivity under mild reaction conditions[3,4]. The bioconversion of sugars into biodiesel occurs through oleaginous yeasts which have been identified as very promising organisms due to their capacity to accumulate up to 90% of their dry weight as lipids, their relatively fast growth rate and ability to grow at high cell densities also on pentose sugars present in the hydrolysates[5]. In this work, the two-steps chemical hydrolysis of giant reed and cardoon was carried out in a microwave reactor employing for the first step both homogenous ($FeCl_3$) and heterogeneous (Amberlyst70) catalysts to recover pentose sugars and diluted sulfuric acid in the second step to recover the glucose.

In order to optimize the synthesis of sugars, the main process parameters were investigated through a chemometric approach: Placket-Burman design of experiments was implemented to define the most important variables and then the response surface methodology analysis was assessed to optimize them. In the future, the synthetized hydrolysate will be employed as substrate in the bioconversion to lipids, through oleaginous yeasts, in order to realize a new route for the production of biodiesel, joining chemical and biochemical approaches.

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Chitosan and chitosan-derivative gels as potential platforms for cellular mechanotransduction

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The conversion of mechanical information from the external microenvironment into intracellular biochemical signaling is termed "mechanotransduction".[1],[2] In this talk I will introduce tridimensional chitosan and chitosan-derivative gels endowed with different strength and elasticity as potential mechanotransducer for cells. Chitosan gels are synthetized through a controlled external ionic gelation using tripolyphosphate (TPP) as cross-linker.[3] Recently it was reported that the physical-chemical properties of chitosan - as well as the molecular weight and the frequency of two building sugars glucosamine (D unit) and N-acetyl-glucosamine (A unit) along polymer chain - play a key role for the setting up of cylindrical gels characterized by a different mechanical behavior if undergone to small/large deformations.[4] Specifically, it was found that the transition from rigid and brittle to weak and much more elastic networks is now possible to achieve simply choosing the proper starting polymer. On the other side, the ability of chitosan gels to behave as scaffold for cellular infiltration and ensuing anchoring was recently demonstrated.[5] Intriguingly, it seems that chitosan gels are suitable milieus for cellular colonization and adhesion without the need of integrin binding motifs - as well as RGD peptides - conversely pivotal in the case of other polysaccharide systems, e.g. alginate-based gels. On the other hand, a lactose-modified chitosan named Chitlac was recently studied for its interesting mechanical properties.[6],[7] Specifically, it was found that Chitlac in the presence of boric acid as cross-linker was able to form transient cross-links if strained at large deformations. Strikingly, its mechanical profile overlapped almost perfectly that of proteins composing the natural extracellular matrix. Overall, the present talk would recapitulate all recent findings on the chitosan-TPP and Chitlac-boric acid gelling systems, with special emphasis on their possible role as platforms for cellular mechanosensing.

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ADDRESS THE CHALLENGE OF THE DEVELOPMENT OF SUSTAINABLE MATERIALS BY EXPLOITING ILS AND POLYSACCHARIDES

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Ionic liquids (ILs) are salts composed of an organic cation and an organic or an inorganic anion which are liquid at or near room temperature. ILs have attracted a great deal of interest in the last 20 years due to some unique physicochemical properties such as the large electrochemical window, the chemical and thermal stability, the negligible volatility, and the no flammability, to mention a few. Polysaccharides such as cellulose, chitosan and chitin are highly abundant renewable feedstocks and represent a potential solution (and challenge at the same time) for the replacement of traditional fuel and plastic material. ILs are typically used for polysaccharides processing and functionalisation due to their unique physicochemical properties, and to their capability to dissolve them [1].

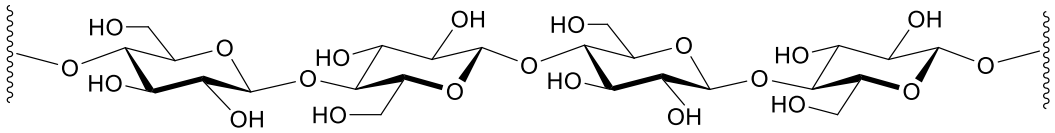

Figure 1. Structure of cellulose.

Indeed, ILs are one of the few solvents able to disrupt the native hydrogen bonds present within the biopolymers,[1] thus allowing their dissolution and further modification. [2]
Herein, we report the development of new bio-based ionic liquids through an easy, single step, environmentally friendly procedure, which has the potential to be scaled-up. Three different classes of ILs, showing a remarkable ability of dissolving cellulose, will be discussed. The chemical modification of chitosan promoted by ILs, which allowed for the obtainment of new bio-based materials, will be also presented.

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SYNTHESIS OF HEXARHAMNOSE-CRM$_{197}$ CONJUGATE AND ITS IMMUNOGENICITY AGAINST GROUP A STREPTOCOCCUS POLYSACCHARIDE

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Group A Streptococcus (GAS) causes autoimmune responses that can lead to rheumatic heart disease and, consequently, to the death of about 350,000 people worldwide annually.[1] GAS clinical isolates express the Group A carbohydrate (GAC), a conserved surface polysaccharide comprising of α-L-Rha*p*(1→3)-α-L-Rha*p*(1→2)-β-D-Glc*p*NAc-(1→3) repeats. Although the main driver of GAS autoimmune responses is the so called M protein; some studies have linked GlcNAc side chain of GAC with cross-reactive epitopes that might also be responsible for rheumatic diseases.[2] On the other hand, it has been shown that the polyrhamnose backbone alone promotes opsonophagocytic killing of multiple GAS strains[3] and, therefore, could be an alternative to GAC for a broadly conserved vaccine candidate against GAS infections. Synthetic GAC oligosaccharides, conjugated to CRM$_{197}$ carrier protein, have been tested as vaccine antigens in a mouse challenge model showing similar immunoprotection to conjugates of native GAC.[4] Two repeating units (hexamer structure) have been reported as the minimal GAC epitope that could secure efficient opsonophagocytosis and protection[4,5].

In this study, a hexarhamnose oligosaccharide was synthesised and conjugated to CRM$_{197}$ carrier protein. Mice were immunised with hexarhamnose and GAC polysaccharide conjugates in order to confirm the use of GAC polyrhamnose backbone as potential GAS vaccine candidate.

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ENGINEERING OF BIOMOLECULAR SYSTEMS FOR ANTI-TUMORAL IMMUNOTHERAPY

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Cancer immunotherapy remains a growing research field. Recent studies [1] have highlighted the interest of antibody recruiting molecules (ARM) to stimulate immune destruction of cancer cells. These bi-functional modules present on the one hand a tumor binding module (TBM) and on the other hand an antibody binding module (ABM) with the aim to highjack endogen antibodies, naturally present in the blood stream, against tumor cells; both are based on peptide-clusters of carbohydrates. Among higher-valency scaffolds, RAFT (Regioselectively Addressable Functionalyzed Template) represents a particularly appealing structure not only for its versatility in terms of orthogonal functionalization but also because has proven to be well tolerated in vivo and non-immunogenic [2]

In our group, we have recently designed new generation of ABMs based on peptide scaffolds (Figure 1) which display clusters of sugars specific for endogen antibodies, i.e. rhamnose [3]. These compounds have been prepared using chemoselective ligations such as azide-alkyne cycloaddition and oxime ligation, and present different, valency, architecture and spacers [4]. These structures will be characterized rigorously by 1D/2D NMR, MS and CD to confirm their molecular definition and interections will be evaluated by microarrays. Complete in vitro and in vivo immunological assays will finally be performed.

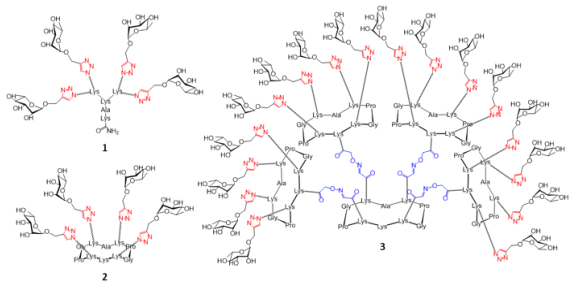

Figure 1. Multivalent glycoclusters functionalized with L-Rhamnose and used as ABMs.

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O-ANTIGEN GMMA-BASED VACCINE AGAINST NONTYPHOIDAL *SALMONELLA*

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Invasive nontyphoidal *Salmonella* (NTS) disease is a leading cause of death and morbidity in Africa. The most common pathogens are *Salmonella enterica* serovars Typhimurium and Enteritidis. The O-antigen portion of their lipopolysaccharide is a target of protective immunity and vaccines targeting O-antigen are currently in development [1]. In the last years, outer membrane vesicles have attracted a lot of attention for the development of vaccines against bacterial pathogens. Extracellular vesicles can be obtained in high yields by genetic mutations, resulting in Generalized Modules for Membrane Antigens (GMMA). We are investigating the use of GMMA as delivery system for *S.* Typhimurium and *S.* Enteritidis O-antigen [2]. We have set up a panel of analytical methods to characterize GMMA as particles, by looking at size, integrity and purity, and to investigate the fine structure of the lipopolysaccharide component [2-3]. We have verified that genetic mutations introduced to increase blebbing and reduce reactogenicity, by detoxifying the lipid A moiety of lipopolysaccharide, can impact both on O-antigen expression and its structural features. Therefore, a careful characterization is needed to identify the best potential GMMA candidates for inclusion in a vaccine against invasive NTS. When tested in mice, GMMA induced high levels of anti-O-antigen specific IgG functional antibodies, despite variations in density and chain length. However, high O-antigen density on GMMA surface would mean less GMMA administered. Methods to check quality, consistency of production, and stability of GMMA vaccines are of fundamental importance, also to support further development of GMMA-based vaccines and their testing in clinical trials. Many of the methods developed are applicable to the characterization of GMMA from other Gram-negative bacteria.

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SYNTHESIS OF II^{3}Neu5Ac-[*6*-^{3}H*Gal*]Gg$_4$-N$_3$

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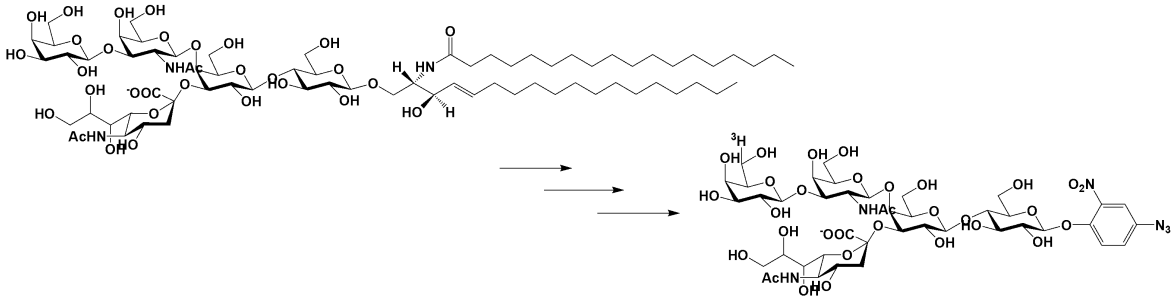
Several data suggest a specific role of ganglioside GM1 in neuronal differentiation and development, but the molecular mechanism of these processes is still unknown. The involvement of GM1 ganglioside in the process of neurite production has been reported for many years. But, recently we showed that the sole oligosaccharide portion of GM1 is responsible for the process of neurite elongation. To continue our studies, we developed a process to synthesize tritium labelled and photoactivable GM1 oligosaccharide

Starting from natural GM1, we obtained the oligosaccharide portion by ozonolysis, than we introduced tritium on external galactose of the oligosaccharide chain.



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NOVEL INSIGHTS INTO N-GLYCANS RECOGNITION BY HOST PROTEINS

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N-glycosylation is the most common post-translational modification present on proteins. The N-glycans, sharing a common pentasaccharide core linked to an asparagine residue, exhibit strtuctural variations associated with tissue and development, but also with diseases, which make them attractive biomarkers. The recognition of cell surface N-glycans by proteins belonging to the host immune system, including Dectins and Siglecs,[1] play a versatile role in human physiology, mediating a wide range of biological processes (Figure 1). Interestingly, aberrant N-glycans – host immune proteins interactions have been associated with an increasing number of pathologies, including infections, autoimmunity and cancer and, therefore, they represent an emerging target to prevent or counteract the course of several disease.

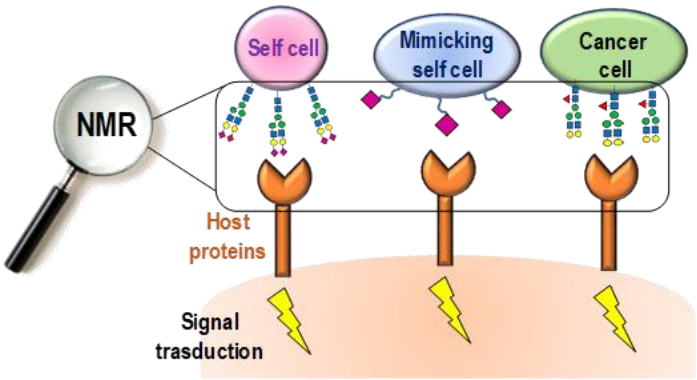

Figure 1. Different mechanisms of interactions between host proteins and cell surface N-glycans.

Within this frame, we are currently investigating the binding between such host immune proteins and different sialylated glycans, both natural occurring and *ad hoc* synthesized,[2] by means of NMR spectroscopy coupled to computational techniques.[3] From such an approach, we could map the interacting epitope, define the ligand bioactive conformation and model the substrate in the protein binding site; these are crucial information, particularly relevant for the development of novel inhibitors or cell-directed therapeutics.

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O-ANTIGEN CHARACTERIZATION ON GENERALIZED MODULES FOR MEMBRANE ANTIGENS (GMMA) FROM *SHIGELLA FLEXNERI* 6

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Shigella infections are one of the top causes of moderate and severe diarrhoea throughout the world and *Shigella flexneri* is the predominant species in developing countries and in children younger than 5 years [1, 2]. The serotype-specific O-Antigen (O-Ag) moiety of *Shigella* lipopolysaccharide (LPS) has been recognized as a key target for protective immunity [3]. No vaccine is currently available and GVGH is considering Generalized Modules for Membrane Antigens (GMMA) as the key technology for the development of a *Shigella* vaccine. GMMA are outer membrane vesicles naturally released from genetically engineered Gram-negative bacteria [4-6], able to display the O-Ag in its natural context and conformation.

The O-Ag biosynthesis depends on the Wzx/Wzy pathway and involves a first step of sugar polymerization during which the O-Ag chain length is regulated by the Wzz proteins, responsible for unique polysaccharide modal lengths. Group 4 capsules (G4C) are instead high molecular weight surface polysaccharides, also known as 'O-antigen capsules', due to their structural similarity to the O-Ag chain of the LPS. G4C share with the LPS the Wzx/Wzy cluster for the biosynthesis of the O-Ag repeating units, but require an additional G4C operon for the assembly of the O-Ag polysaccharide into the capsular structure.

In the present study, *S. flexeneri* serotype 6 was engineered to obtain an hypervesiculating phenotype by removing the *tolR* gene. Resulting GMMA showed to display a medium molecular weight O-Ag population with average size of 23 kDa and a high molecular weight G4C with average size of 175 kDa. Both polysaccharide species shared the expected structure, consisting of a tetrasaccharide repeat containing one N-acetylgalactosamine (GalNAc), one galacturonic acid (GalA) and two rhamnose residues (Rha^{II}-Rha^{III}), with Rha^{III} being variably O-acetylated. The strain was therefore further mutated in order to abolish the capsule formation, by removing the *ept-etk* genes in the G4C operon, and to prevent O-Ag chain length regulation by removing the *wzzB* gene. This resulted in the presence of O-Ag chains with few repeats only.

In conclusion, we developed analytical methods for the full characterization of *S. flexneri* 6 GMMA, with particular attention to the O-Ag component, and we obtained GMMA from a set of *S. flexneri* 6 mutants differing in polysaccharide length and density. How these modifications can impact the immune response will be investigated. The overall goal of this study will be to demonstrate the utility of engineering Gram-negative bacteria to produce homogenous O-Ag populations as the basis for GMMA-based vaccines.

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IMINOSUGAR-BASED TREHALASE INHIBITORS: THE KEY ROLE OF LINKER LENGTH AND FLEXIBILITY

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Recently, the need for selective inhibitors towards insect trehalase emerged. The specificity of new insecticides is crucial to avoid potential toxic effects for plants and mammals, but also for insects that are benefit in nature[1]. The role of trehalose (α-D-glucopyranosyl α-D-glucopyranoside) in insects is related to its hydrolysis operated by the enzyme α-trehalase (EC 3.2.1.28), that cleaves the glycosidic bond converting trehalose into two units of glucose.[2] Among the most powerful inhibitors of trehalases, there are some natural pseudodisaccharides and analogues, such as validoxylamine, casuarine-6-*O*-D-glucoside and some non-natural analogues.[3]

In this work the synthesis of new pseudodisaccharide mimetic **1**, using a stereoselective α-glucosylation is presented. The derivative, bearing a glucosyl moiety and a pyrrolizidine portion, was screened as inhibitor of insect trehalase from *C. riparius*.. The decrease of potency and selectivity towards the insect trehalase with respect to previous reports[4] showed the key role played by the stereochemical configuration at C-6 of the pyrrolizidine nucleus.

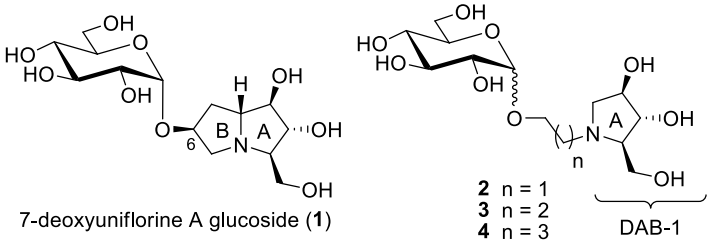

Figure 1. New pseudodisaccharide mimetic **1** and a series of more flexible disaccharide mimics **2-4**.

Moreover, a simpler synthetic strategy was developed and allowed to obtain new pseudodisaccharide insect trehalase inhibitors with a pyrrolidine core (with both α and β anomeric configurarations). These results demonstrated the pivotal function played by the distance between the glucosyl and the iminosugar pyrrolidine moiety in these flexible inhibitors, as well as by the anomeric configuration of the glucose moiety.[5]

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DE NOVO SYNTHESIS OF L-DNJ AND ITS *N*-ALKYLATED DERIVATIVES

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Iminosugars (sugar analogues with an amino function in place of the endocyclic oxygen) represent the most important class of glycomimetics.[1] Due to their ability to inhibit or increase the activity of carbohydrate-processing enzymes, iminosugars possess a high pharmacological potential, even though their therapeutic applications are hampered by a weak selectivity.[2] With the aim to overcome this problem, the role of iminosugar configuration has been explored over the last years.[2] Exploiting the lack of stereoselectivity of specific, biologically important glycosidases,[2] L-iminosugars and their *N*-alkylated derivatives have been used as selective enzyme inhibitors[2]/enhancers,[3] surpassing in some cases the pharmacological potential of their D-enantiomers.[3]

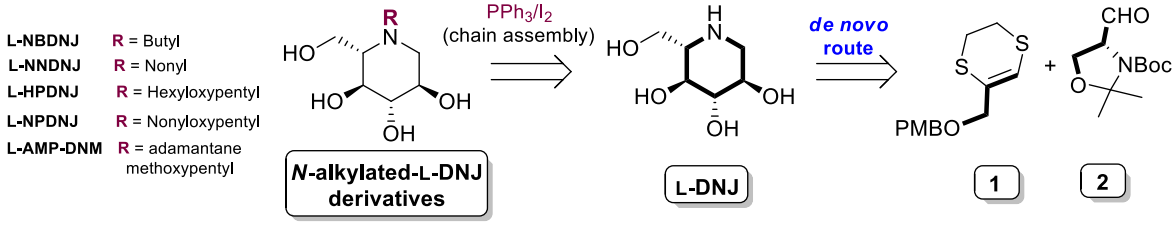

FIGURE 1 *De novo* route to L-DNJ and its *N*-alkyl derivatives.

Based on these findings, herein we report a synthetic procedure for the preparation of L-DNJ and its *N*-alkylated derivatives (**FIGURE 1**). The highly stereocontrolled access to the iminosugar core has been devised through *de novo* synthesis starting from the synthetically available homologating agent **1**[3,4] and the Garner aldehyde **2**. In addition, the use of polymer-bound triphenylphosphine/iodine has been conceived for the assembly of the alkyl chains, thereby providing a variety of *N*-alkyl L-DNJ derivatives. Biological assays for some of these derivatives are also presented, revealing an interesting therapeutic potential in the treatment of some genetic disorders including Pompe disease[3] and Cystic Fibrosis.

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SYNTHESIS OF GLYCOCONJUGATE H_2S-DONORS

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Hydrogen sulfide (H_2S) is a well-known gaseous signaling molecule, which displayed anticancer properties, as well as other important roles in many physiological processes. The design of new H_2S-releasing molecules generally aims at the selective and controlled H_2S delivery toward the target site, depending on the condition to be treated.[1] In this respect, our strategy was to synthetize various glycoconjugated H_2S-donors,[2] where glycopyranoside portions are bound to well-known H_2S donors, such as ADT-OH[3] or aryl/alkyl isothiocyanate moieties (Figure 1).[3] The synthesis of these new H_2S-releasing glycoconjugates was implemented and the resulting products were eventually tested *in vitro* in pancreatic cancer cells, to assess their inhibition of the cell viability and their ability to release H_2S in the cytoplasm.

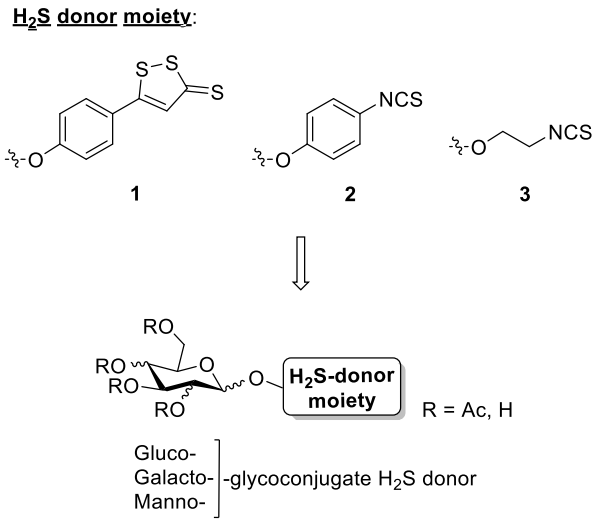

Figure 1: H_2S-donor moieties and general glycoconjugate structure.

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ONE-POT SYNTHESIS OF PSEUDO-THIODISACCHARIDES THROUGH AZIRIDINE OPENING REACTIONS

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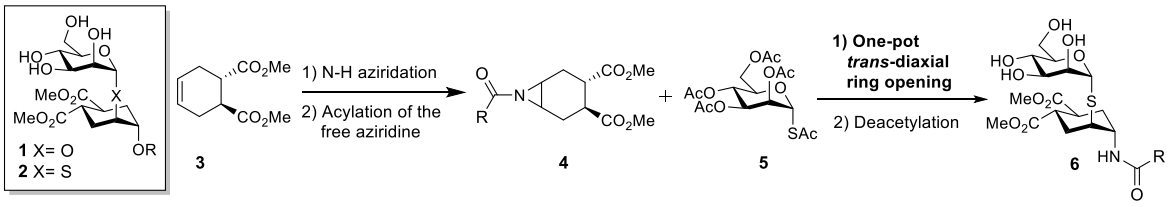
Glycomimetics are under active investigation as antagonists of medically relevant lectins implicated in cell-cell communication and pathogen recognition events. Indeed, carbohydrate mimics can be designed as competitors of natural lectin ligands and they often show improved drug-like characteristics and stability to enzymatic degradation.
Our group has been developing selective DC-SIGN ligands based on the structure of the pseudo-1,2-dimannoside **1**.[1] Recently we have also reported an efficient synthesis of the corresponding pseudo-*thio*-1,2-dimannoside **2**.[2] Since they are tolerated by most biological systems, thioglycosides and derivatives are attracting significant interest in the development of new therapeutics. In particular compound **2** was found to share with **1** both the conformational behavior and the DC-SIGN binding activity, but displayed an improved resistance to enzymatic hydrolysis by α-mannosidase (jack bean), thanks to the presence of the sulfur-linkage.
We now report conditions for the one-pot ring-opening reaction of aziridine **4** by α-mannosyl thiolate, generated *in situ* by a selective *thio*-deacylation of **5**. Starting from the enantiomerically pure olefin **3**, the free aziridine was prepared[3] and coupled *in situ* with various functionalized or un-functionalized acylating agents, to give *N*-acylaziridines of general structure **4**. Finally, the pseudo-*thio*-disaccharide **6** was obtained as a single isomer by an exclusively *trans*-diaxial opening of **4** by the α-thiolate of **5**, followed by deacylation of the sugar moiety.
All the synthetic and mechanistic aspects will be discussed, as well as preliminary data about the scope of the approach. Furthermore, the affinity of the *thio*-glycomimetics **6** towards DC-SIGN, as determined by SPR analysis, will be presented.



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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreements No 642870 (Immunoshape) and No. 765581 (PhD4GlycoDrug).

SYNTHESIS OF MENA HYDROLYTICALLY STABLE ANALOGUES FOR IMPROVED ANTI MENINGOCOCCAL VACCINE

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The *Neisseria meningitidis* type A (MenA) is an encapsulated Gram negative bacterium. Among the 12 serogroups identified, the serotype A was a major cause of meningitis in developing countries and particularly in the sub-Saharan region of Africa called "meningitis belt" [1]. Glycoconjugate vaccine based on the capsular polysaccharide tackling this pathogen are now available [2]. However the development of a liquid glycoconjugate vaccine is challenging because of the poor stability in water of the natural capsular polysaccharide (CPS). This is due to the phosphodiester linkages holding together the (1-6)-2-acetamido-2-deoxy-α-D-mannopyranosyl repeating units forming the capsular polysaccharide (fig. 1. A). To fix this lability, we envisaged two strategies based on the replacement of *i*) the pyranose ring oxygen (*carba* analogues, fig 1. B), [3] and *ii*) the anomeric oxygen (phosphono analogues, fig. 1. C) [4] by a methylene group.
Thus, we describe our synthetic approaches to *carba* analogue and phosphono analogue of N-acetyl mannosamine (the repeating unit of MenA CPS). Combined in silico/NMR studies have been used to understand the mimicry of the analogues with the natural sugars and enlighten their conformational characteristics.

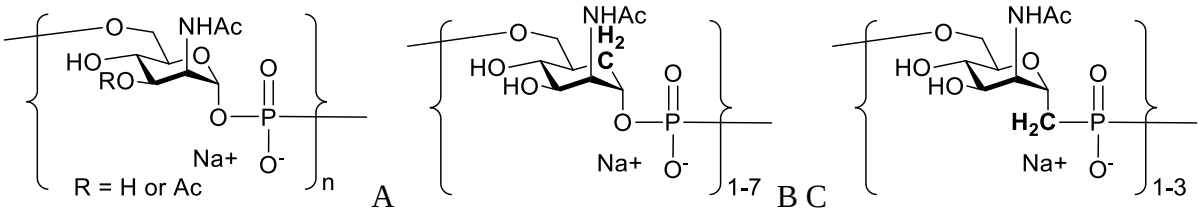

Figure 1. (A) N-acetylmannosamine-(1-α-O-phosphate), the repeating unit of MenA polysaccharide; (B) Phosphodiester-linked *carba* oligomers; (C) Phosphonoester-linked oligomers.

We will describe the synthesis of *carba* and phosphono analogues of MenA CPS oligomers. In addition, *carba* analogues up to trimer were conjugated to CRM_{197} as a protein carrier, and the immunological profile of the resulting *neo*-glycoconjugates was carefully investigated.[5]

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[6]. This project has received funding from the H2020-MSCA-ITN-2015 "Glycovax" under grant agreement No 675671.

DESIGN AND SYNTHESIS OF MODIFIED GLICOAMINO OPEs FOR THE FINDING OF NEW POTENTIAL PHOTOSENSITIZERS

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There are several features that a fluorescent molecule must have to be used as probe in medical imaging, among them the biocompatibility and the water solubility are essential. Usually, when small fluorophores are conjugated with a sugar or an amino acid, this last characteristic is satisfied.[1] In particular, carbohydrates' role ranges from energy storage molecules to structural constituents of cells and tissues. Furthermore, the semimobile heterocycle of natural D-monosaccharides offers the conditions to use them as flexible spacers in the construction of more complex, luminescent architectures.[2]

Recently, we reported the synthesis of end-only glucose functionalized Oligo(phenylene-ethynylene)s (OPEs) where the balanced contribution of the hydrophilic (sugar) and hydrophobic (aryl conjugated system) moieties gives rise to the permeation of some of these OPEs to the cellular membrane, showing their potential uses as dyes in fluorescent imaging microscopy. Furthermore, the presence of the dimethylamino group is responsible of the generation of singlet oxygen, opening the way in their use as Photosensitizers.[3]

Going on with our research, and in order to explore the possibility to improve their use in the medical and biological fields, our aim has been to synthesize new OPEs, by changing the substituents at the central chain and by introducing heavy atoms that can improve the singlet oxygen production (Heavy Atom Effect). Moreover, a new modular approach has led to the desymmetrization of the chain, with the aim of anchoring our biocompatible systems to upconverting lanthanide nanoparticles The first results of this new research, starting from the synthesis of model compounds, will be the subject of this communication.

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SYNTHESIS AND ICE-RECRYSTALLIZATION INHIBITION EVALUATION OF THE TETRASACCHARIDE REPEATING UNIT OF THE CRYOPROTECTANT CAPSULAR POLYSACCHARIDE FROM *Colwellia psychrerythraea* 34H

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Colwellia psychrerythraea 34H, a Gram-negative psychrophilic bacterium isolated from Arctic marine sediments at -1°C, provides a model for the study of life in cold enviroments. Its cryotolerance is due to the production of a unique capsular polysaccharide (CPS) with anti-freeze properties similar to the well-known anti-freeze (glyco)proteins. [1] Such CPS consists of a tetrasaccharide repeating unit containing two aminosugars (*N*-acetyl-glucosamine, GlcNAc and *N*-acetyl-galactosamine, GalNAc) and two uronic acids (glucuronic acid, GlcA, and galacturonic acid, GalA), linked in an alternating fashion, with a threonine (Thr) as a substituent. [2] In order to explore in detail the relationship between structure and ice-recrystallization inhibition activity, it has recently been launched a project aimed to the synthesis of oligosaccharide fragments of such CPS. The tetrasaccharide repeating unit was synthesized as an *O*-*n*-propyl glycoside starting from appropriate monosaccharide and aminoacidic building blocks, which have been then assembled in a regio- and stereoselective manner. The synthesis faced with some challenging features such as building up a crowded [→2)α-D-Galp(1→] moiety as well as differentiating the two uronic units for the regioselective insertion of Thr amide only on one of them. The obtained tetrasaccharide was firstly analyzed by NMR to confirm the structure proposed for the CPS from *C. psychrerythraea* and then subjected to ice-recrystallization inhibition (IRI) evaluation to determine if it actively interacts with ice.

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DECODING AKKERMANSIA MUCINIPHILA LIPOPOLYSACCHARIDES

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Akkermansia muciniphila is a commensal non-pathogenic bacterium that inhabits the mucus layer of mammals' guts. This mucus layer is a physical defense that covers and protects the intestinal epithelium against pathogens and chemicals. Mucus contains antibacterial peptides and antibodies; even so, this non-pathogenic bacterium is one of the few that successfully inhabits there [1,2]. Its integrity depends on diverse homeostatic regulatory mechanisms where microbiota-host interactions play a crucial role [3]. *Akkermansia* participates as a mucin degrader, breaking down the main glycoprotein of the mucus layer. This way, it is a fundamental actor in the renovation and well being of the mucus layer [4]. Also, there is diverse evidence of its capacity to perform important changes beyond the intestine. In metabolic diseases such as obesity and diabetes, *Akkermansia* population decreases, producing thinning mucus layer and inflammatory processes [5,6].

As gram-negative bacteria, *Akkermansia* possesses lipopolysaccharides (LPS) as mayor components of its exterior membrane, which protects the bacteria and interacts with the environment and the host [7]. Recent studies suggest that LPS can produce relevant changes on the intestinal epithelium permeability and integrity, although the mechanisms are not clear [8].

Identifying the composition and particularities of the LPS is crucial to understand the details of this mutualism relation, also its capacity to live in the mucus layer and to participate in metabolic disorders. Through a multi-technique approach comprising chemical analyses and diverse spectroscopy techniques, the chemical composition and structure of *Akkermansia muciniphila* lipopolysaccharides was determined. Globally, our results shed light on the molecular basis of the role of LPS on the mutualism between humans and *A. muciniphila*. Nevertheless, future in vitro and in vivo studies should be warranted to progress in the understanding on this special mutualism relation.

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STRUCTURE OF LIPOOLIGOSACCHARIDES FROM HALOPHILIC BACTERIA

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Extremophiles are organism that can live in harsh environment. They can produce unique metabolites as they adapt to their particular conditions and are hence an interesting source of biologically active compounds [1]. Directly exposed to their environment, Lipopolysaccharide (LPS) and Lipooligosaccharide (LOS) are the main component of the outer membrane of Gram-negative bacteria. LPSs are composed of three main parts: a polysaccharide named the O-antigen, a core oligosaccharide and the Lipid A that is anchored in the outer membrane. These endotoxins are known to interact with mammal's innate immunity, being agonist or potential antagonist of the Toll-Like Receptor (TLR4) and Myeloid Differentiation factor 2 (MD-2). Depending on their structures, endotoxins can be potent modulators of TLR4/MD2, leading to potential therapies [2]. In the case of extremophiles, LPS can possess unusual structure and immunological activities [3]. New structural features can then be found in halophiles, organisms that live in high salinity conditions.

In this context, endotoxins from different halophiles were characterized. *Spiribacter salinus* is a gamma-proteobacterium isolated from intermediate-salinity pound in Spain [4]. Its Lipid A was characterized using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) and MS^2 techniques [5]. The structure of *Halopeptonella vilamensis* LOS was also investigated. It is a gamma-proteobacterium isolated in a saline lagoon in Argentina [6]; its Lipid A was analysed by MALDI-MS and MS^2 and its oligosaccharide content was studied with NMR spectrometry.

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CHEMICAL STRUCTURE OF LIPOPOLYSACCHARIDES ISOLATED FROM *VEILLONELLA PARVULA*

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The human gut microbiota harbours a complex community of microorganisms which influences human physiology, metabolism, nutrition and immune function. To remain immunologically tolerant to these commensal bacteria and preserve this symbiotic relationship, elaborate biochemical mechanisms exist. Bacterial cell wall glycoconjugates such as Lipopolysaccharides (LPS) act as microbe-associated molecular patterns (MAMPS) and are vital for the initiation of immune response to pathogens as well as immunological suppression to symbiotic bacteria. The aim of this research project is the extraction, purification and structural elucidation, through Mass Spectrometry and NMR techniques, of LPS isolated from commensal bacterial species such as *Veillonella parvula*. *V. parvula* has been found to play a vital role in poly-microbial infections specifically in respiratory and oral infections. Conversely, alternative research has shown that its LPS shows a protective behaviour towards the effects of toxic LPS and can influence the susceptibility of children to allergies and autoimmunity. Therefore, *V. parvula* is a key component of a healthy microbiota and since LPS is involved in the interaction between the bacteria and the host, the investigation of the LPS structure is necessary to shed the light on the molecular mechanisms at the basis of the beneficial effects of commensal bacteria.

DECIPHERING THE RECOGNITION PATTERNS OF LECTINS INTERACTION WITH GLYCAN MOTIFS OF LIPOPOLYSACCHARIDES USING NUCLEAR MAGNETIC RESONANCE (NMR)

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The bacterial cell envelope constitutes the interface with the external environment and it is the first site of contact between microbes and their hosts. The surface of Gram-negative bacteria is decorated by amphiphilic macromolecules, known as lipopolysaccharides (LPS) (or endotoxins), whose presence is required for bacterial survival, growth and interaction with any other cell [1] (Figure 1). LPSs expose to the outside highly complex and strain-dependent glycan motifs recognized by a family of carbohydrate binding proteins called lectins. The aim of this work is to decipher the recognition patterns of LPSs in the interaction with lectins. With this aim we will i) produce human and bacterial lectins, ii) extract and purify LPS from bacterial cells[2,3], iii) analyze and characterize their interactions by using NMR techniques[4,5]. This project will be shared between the University of Naples and the university of Grenoble (France).

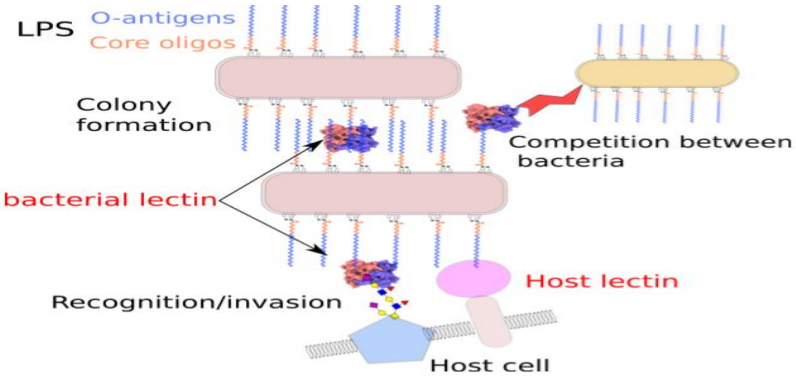

Figure 1: Different types of bacteria and host lectins communications mediated by LPS.

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ANALYSIS OF THE STRUCTURAL BASIS OF *O*-ACETYL EPITOPES AS KEY COMPONENTS OF THE IMMUNOGENICITY OF VI POLYSACCHARIDE

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Typhoid fever caused by *Salmonella* Typhi represents a serious public health concern in developing countries, affecting millions of people each year, especially young children[1]. Vi polysaccharide (Vi PS) forms a capsule around *S.* Typhi and is both a virulence factor and a protective antigen[2]. Anti-Vi PS antibodies confer protection against infection and Vi PS is currently licensed as a vaccine against typhoid fever[3]. Vi PS is a linear homopolymer of poly-α(1→4)-D-GalANAc with a 60-100% level of *O*-acetylation at the C-3 position. The *O*-acetyl groups at C-3 are reported to make up most of the surface of Vi PS and bind anti-Vi antibodies, as the immunogenicity of Vi PS is known to be closely related to its degree of *O*-acetylation[4]. The presence of *O*-acetyl groups may well be associated with the intrinsic viscosity of Vi PS solutions[5]. In this study, the effect of *O*-acetylation on Vi PS has been investigated in detail. Antibody binding studies using variably de-*O*-acetylated Vi PS obtained from *S.* Typhi and *Citrobacter freundii* following exposure to several levels of ammonium hydroxide confirmed the immunodominance of *O*-acetyl groups over other epitope(s) on de-acetylated Vi PS. Molecular dynamics simulations showed that a fully acetylated Vi PS has little mobility and that *O*-acetyl groups lock the saccharide chain into a helix by blocking rotations of neighbouring residues. On the other hand, increasing levels of de-*O*-acetylation produced a more flexible polymer chain and solution of lower viscosity.
These studies contribute to a better characterization of Vi PS and may lead to optimization of the production of Vi PS-based reference materials and vaccines.

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O-ANTIGEN CHARACTERIZATION ON GENERALIZED MODULES FOR MEMBRANE ANTIGENS (GMMA) FROM *SHIGELLA FLEXNERI* 6

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Shigella infections are one of the top causes of moderate and severe diarrhoea throughout the world and *Shigella flexneri* is the predominant species in developing countries and in children younger than 5 years [1, 2]. The serotype-specific O-Antigen (O-Ag) moiety of *Shigella* lipopolysaccharide (LPS) has been recognized as a key target for protective immunity [3]. No vaccine is currently available and GVGH is considering Generalized Modules for Membrane Antigens (GMMA) as the key technology for the development of a *Shigella* vaccine. GMMA are outer membrane vesicles naturally released from genetically engineered Gram-negative bacteria [4-6], able to display the O-Ag in its natural context and conformation.
The O-Ag biosynthesis depends on the Wzx/Wzy pathway and involves a first step of sugar polymerization during which the O-Ag chain length is regulated by the Wzz proteins, responsible for unique polysaccharide modal lengths. Group 4 capsules (G4C) are instead high molecular weight surface polysaccharides, also known as 'O-antigen capsules', due to their structural similarity to the O-Ag chain of the LPS. G4C share with the LPS the Wzx/Wzy cluster for the biosynthesis of the O-Ag repeating units, but require an additional G4C operon for the assembly of the O-Ag polysaccharide into the capsular structure.
In the present study, *S. flexeneri* serotype 6 was engineered to obtain an hypervesiculating phenotype by removing the *tolR* gene. Resulting GMMA showed to display a medium molecular weight O-Ag population with average size of 23 kDa and a high molecular weight G4C with average size of 175 kDa. Both polysaccharide species shared the expected structure, consisting of a tetrasaccharide repeat containing one N-acetylgalactosamine (GalNAc), one galacturonic acid (GalA) and two rhamnose residues (Rha^{II}-Rha^{III}), with Rha^{III} being variably O-acetylated. The strain was therefore further mutated in order to abolish the capsule formation, by removing the *ept-etk* genes in the G4C operon, and to prevent O-Ag chain length regulation by removing the *wzzB* gene. This resulted in the presence of O-Ag chains with few repeats only.
In conclusion, we developed analytical methods for the full characterization of *S. flexneri* 6 GMMA, with particular attention to the O-Ag component, and we obtained GMMA from a set of *S. flexneri* 6 mutants differing in polysaccharide length and density. How these modifications can impact the immune response will be investigated. The overall goal of this study will be to demonstrate the utility of engineering Gram-negative bacteria to produce homogenous O-Ag populations as the basis for GMMA-based vaccines.

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ATOMIC FORCE MICROSCOPY INVESTIGATION OF THE BIOFILM POLYSACCHARIDE FROM *Burkholderia multivorans* C1576

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Biofilms (BF) are the most common mode of life of bacteria. To setup biofilms bacteria adhere to a surface and biosynthesize a macromolecular matrix which immobilizes cells and useful molecules (enzymes and nutrients). The major BF components are exopolysaccharides (Epols) which constitute the gel-like matrix typical of biofilms. BF are relevant in environmental and industrial settings, as well as in disease, since BF-associated infections are very difficult to eradicate. Therefore, the definition of the mechanisms involved in matrix stability and the role of matrix components in BF functions are particularly important. We investigated the structure of Epols extracted from biofilms of different strains of the *Burkholderia cepacia* Complex bacteria, a family of opportunistic pathogens causing serious lung infections. Among them, the one produced by *B. multivorans* strain C1576 (EpolC1576) attracted our attention for the presence of Rha sequences and of -OCH_3 groups on 25% of Rha units. These features suggested a rather "hydrophobic" nature of the Epol backbone as proved by its ability to bind aromatic fluorescent probes[1]. In addition, molecular modeling investigations showed that the EpolC1576 chain is rather flexible[1].

To better clarify the possible role of this Epol in biofilm formation and stability we performed AFM experiments by spray-drying polymer solutions on mica surfaces. The obtained images revealed a spherical shape of the whole polysaccharide chain confirming the flexible nature of the backbone. In addition, at high Epol concentrations AFM showed large aggregates where the packing typical of spherical objects was recognized. The aggregation on one side confirmed the "hydrophobic" nature of the Epol and, on the other hand, suggested its role in biofilm matrix formation.

For comparison, AFM experiments on a different polysaccharide produced by the same strain but in non-biofilm conditions will be discussed.

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SYNTHESIS OF PHOSPHONODISACCHARIDE ANALOGUE FROM *NEISSERIA MENINGITIDIS* A CAPSULAR POLYSACCHARIDE

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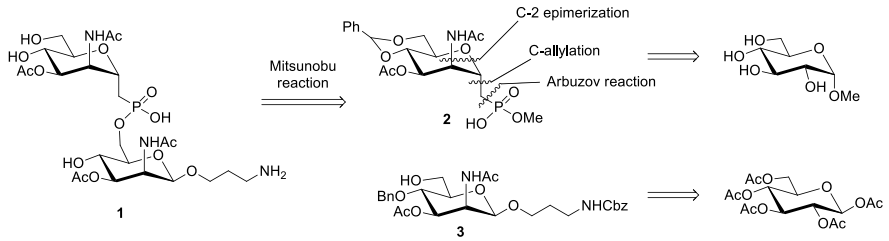
Neisseria meningitidis, a Gram-negative bacterium, is one of the major causes of bacterial meningitis. Serotype A (MenA) is the main responsible for epidemics in sub-Saharan Africa. The structure of MenA capsular polysaccharide consists of (1→6)-linked-2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate residues acetylated at C-3 to an extent of 70-90%. The natural polysaccharide, once isolated, results to be not stable in aqueous formulations due to the intrinsic lability of the anomeric phosphodiester linkages. Isosteric phosphono analogues, previously synthesized by our group, proved to be much more stable to hydrolysis and to be recognised by anti-MenA serum.[1] Since recent studies revealed that 3-*O*-acetylation is crucial for immunogenicity,[2] we decided to synthesize the 3-*O*-acetylated phosphono analogue, focusing our attention on the disaccharide **1** (Scheme 1) due to its intriguing immunogenic properties.[3] The target molecule **1** could be achieved by Mitsunobu coupling of phosphonate **2** with mannoside **3** which bears an amino spacer suitable for conjugation. Acetyl group could be installed at C-3 early in the synthesis. The correct sequence of key transformations (Scheme 1) must be carefully considered to obtain phosphonate **2**. In this communication we describe the strategies we explored for an efficient approach to methylphosphonate **2** as key precursor of target compound **1**.



Scheme 1: *Retrosynthetic strategy*

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"NORTHERN" RIBOSE AND PYROPHOSPHATE MODIFIED cADPR ANALOGUES: A NOVEL CLASS OF POTENTIAL Ca^{2+} MOBILIZERS

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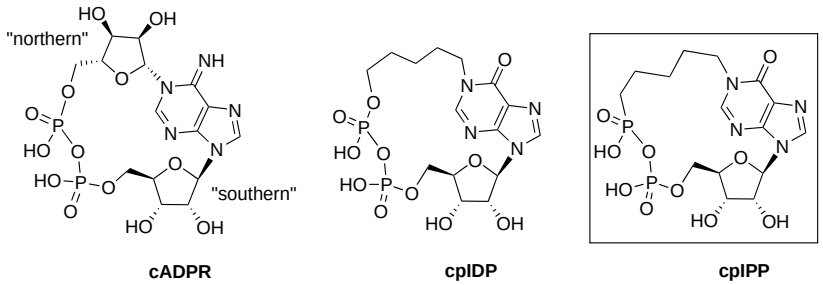
Cyclic ADP-ribose (cADPR) is a natural occurring metabolite of NAD^+ capable of mobilizing Ca^{2+} ions from intracellular stores. It was firstly isolated from sea urchin eggs extract, but it was later established that it is also produced in many other mammalian cells, including pancreatic β-cells, T-lymphocytes, smooth and cardiac muscle cells and cerebellar neurons, acting as a Ca^{2+}-mobilizing agent. For this activity, cADPR has been classified as a second messenger that, activating the ryanodine receptors of the sarcoplasmatic reticulum, is able to mobilize the calcium ions from intracellular stores. cADPR is involved in many physiological processes related to the variation of the Ca^{2+} concentration, such as the synaptic homeostasis in neurons, as well as fertilization and cellular proliferation.



This cyclic nucleotide, characterized by a very labile glycosidic bond at the N1, is rapidly hydrolysed also in neutral aqueous solutions to the inactive ADP-ribose. Matsuda and co-workers[1] were the first who synthesized new analogues of the cADPR in which the adenine base was replaced by a hypoxanthine ring. This kind of modification produced the cyclic inosine diphosphate ribose (cIDPR) which proved to be stable in hydrolytic physiological conditions and showed significant Ca^{2+} mobilizing activity. A lot of modifications regarding the northern and southern ribose, as well as the purine base of cADPR, have been proposed so far. In our laboratories we have synthesized several analogues of cIDPR.
In particular, the analogue with the northern ribose replaced by a pentyl chain (cpIDP) showed interesting Ca^{2+} mobilizing activity on the neuronal PC12 cell line.[2] Starting from these results, we report here the synthesis of the novel analogue cpIPP, in which the "northern" ribose of cADPR was replaced by a pentyl chain and the pyrophosphate moiety by a phophono-phosphate anhydride. The effect of the presence of the new phosphono-phosphate bridge on the intracellular Ca^{2+} release induced by cpIPP was assessed in PC12 neuronal cells.[3]

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THE INFLUENCE OF CONJUGATION VARIABLES ON THE DESIGN AND IMMUNOGENICITY OF A GLYCOCONJUGATE VACCINE AGAINST *SALMONELLA* TYPHI

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Recent years have seen major efforts to develop glycoconjugate vaccines based on the Vi polysaccharide that will protect against *Salmonella enterica* serovar Typhi infections, particularly typhoid fever, which is a major public health concern in low-income countries. Different variables in the design of glycoconjugate vaccines can influence the immune response they elicit. Here we systematically test the response in mice to Vi glycoconjugates that differ in Vi chain length, carrier protein, conjugation chemistry, saccharide to protein ratio, and size.

We found that the length of Vi chain, but not the ultimate size of the conjugate, impacted the anti-Vi IgG immune response elicited. Full-length Vi conjugates, independent of the carrier protein, induced peak IgG responses rapidly after just one immunization, and a second dose did not enhance the magnitude of these responses. Fragmented Vi linked to CRM_{197} and diphtheria toxoid, but not to tetanus toxoid, gave lower anti-Vi antibody responses after the first immunization than full-length Vi conjugates, while antibody titers were similar to those induced by full-length Vi conjugates following a second immunization. The chemistry used to conjugate Vi to the carrier protein, the linker used, and the saccharide to protein ratio did not significantly alter the response.

In conclusion, from this study Vi length and carrier protein are the variables that most influence the anti-Vi IgG response to immunization, while other parameters appear to be of lesser importance.

"*The research leading to these results has received funding from the Poeple Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013 under REA grant agreement No. 316940*".

THE 6-DEOXY HEXOSES METABOLISM IN *TRICHOMONAS VAGINALIS*

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Trichomonas vaginalis is an anaerobic flagellated protozoan human parasite, causative agent of Trichomoniasis. Similarly to bacteria, *T. vaginalis* presents an external membrane characterized by complex poysaccharide structures, where lipophospohoglycan (LPG) is the most abundant (1). LPG is demonstrated to have a crucial role in the interaction between the host and the pathogen and in the consequent infection (1). The LPG glycan structures from *T. vaginalis* are rare and they are not found in other organisms. The main component is L-rhamnose (1); this 6-deoxy exose is common in bacteria, but absent in humans and, for that reason, it could be a pharmacological target (2) (3).

The metabolism of the 6-deoxy exoses is mediated by three fundamental reactions: a dehydratation, an epimerization and a reduction (3). In eukaryotes this type of pathway leads to the production of the L-fucose, starting from the GDP-D-mannose (4); in prokaryotes, other pathways are present for 6-deoxyhexose production, including the dTDP-L-rhamnose pathway starting from the dTDP-D-glucose (3). Plants and lower metazoa can also produce L-rhamnose, using UDP-D-glucose as precursor (5).

In this study we have identified the putative enzymes for L-Rhamnose metabolism in *T. vaginalis*. In particular, we cloned, expressed and characterized the first enzyme involved in this pathway, the UDP-D-glucose 4,6 dehydratase (UGD) and the putative last enzyme of the pathway, a NADPH-depednent 4-keto reductase (RED); no conclusive data were obtained for the 3,5-epimerase, responsible for the conversion from D- to L- configuration of the monosaccharide. This data suggest that the production of the epimerase need to be more investigated. In addition, the study of inhibitors could help to find an alternative healing because the pathogen easily develops resistance to canonical treatments (6).

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STUDY OF THE INTERACTION BETWEEN SIALIC ACID-BINDING IMMUNOGLOBULIN-TYPE LECTINS (SIGLEC) AND SIALYLATED GLYCANS FOR THE DEVELOPMENT OF A NEW GENERATION OF IMMUNOMODULATORS.

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Sialic acid-binding immunoglobulin-like lectins, or Siglecs, are cell surface receptors that recognize sialic acid (Sia) and are known to modulate immune responses, influencing almost every cell in the immune system. Siglecs are involved in events like cell adhesion and signaling, inhibition or regulation of the immune cell activation, all mediated by the interaction with sialylated ligands[1,2]. Sialic acid-Siglec interactions have been associated with a broad spectrum of diseases, ranging from autoimmunity to neurodegeneration and cancer [3,4]. Thus, strategies to tune the interaction between Siglecs and sialylated glycans in pathophysiological processes could have great therapeutic potential. Within this frame, we intend to analyze, at a molecular level, the interaction between Siglecs and their natural and synthetic substrates with the aim to exploit their properties for treatment of human diseases. Indeed, the understanding of the molecular mechanisms at the basis sialoglycans recognition by Siglecs will open a route for the design of novel glycomimetics for therapeutic targeting of the Siglecs – sialylated glycans axis.

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THE INTERPLAY BETWEEN NMR SPECTROSCOPY AND MOLECULAR MODELING: A POWERFUL STRATEGY TO INVESTIGATE PROTEIN-GLYCOCONJUGATE INTERACTIONS.

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Protein-carbohydrates interactions set the basis of molecular recognition processes, indeed, they are involved in events like cell–cell interactions, signal transduction, inflammation, viral entry, and host bacteria recognition, thereby participating in disease, defense and symbiosis[1,2]. Understanding the roles that complex carbohydrate such as glycans plays in the aforementioned biological events offers opportunities for developing therapeutic strategies for the treatment of various diseases[3].

However, unraveling the dynamic of protein- glycoconjugates interactions represents a major challenge due to extreme structural complexity and variability of both mammalian and bacterial glycans, as well as the multivalent nature of their interactions with proteins[4,5]. With the raised awareness that the presentation of a glycan epitope can affect its recognition by proteins[3], the aim of the work is to rigorously define the bioactive conformation and binding epitope of the selected ligand for a deeper comprehension and modulation of biological processes closely related to biomedicine applications[6]. To reach this goal, the protein–glycoconjugate binding events are investigated with magnetic resonance (NMR) techniques, particularly a ligand-based approach is applied[7]. Furthermore, computational methods are implemented to provide a complete picture of protein- glycoconjugate binding mechanisms.

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NMR STUDY OF INTERACTIONS BETWEEN SIGLECS AND SYNTHESIZED COMPLEX-TYPE *N*-GLYCANS: THE CASE OF SIGLEC-2

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Sialic acid-binding Immunoglobulin (Ig)-like lectins (*Siglecs*) constitute a family of transmembrane glycan-binding receptors that decorate immune cells surfaces and are implicated in both innate and adaptive immunity. These lectins recognize and bind sialic acids containing glycans, which represent a common structural motif exposed on all mammalian cells. Siglecs perform many regulatory roles and are involved in events like cell-cell communication, inhibition or regulation of immune tolerance and also in host-pathogen interactions.[1] When an aberrant interaction occurs between a Siglec and the cognate sialylated ligand, a possible variety of pathologies, including infections, autoimmunity, and even cancer, may occur. This work has been focused on the study of the interactions between Siglec-2, also known as CD22, and *ad hoc* synthesized complex-type *N*-glycans by means of NMR binding experiments. Siglec-2 is predominantly expressed on B cells and acts as inhibitory receptor of B cell antigen receptor (BCR) signal, inducing tolerance to self-antigens, thus avoiding autoimmune processes. NOE-based NMR techniques were used to obtain information about synthesized complex-type *N*-glycans in their free state. Furthermore, advanced NMR methods, including STD (Saturation transfer Difference) NMR and transferred NOE (trNOE), were used to characterize the binding mode of the sialylated ligands to CD22 as well as to deliver the conformations of the ligands in the bound state.[2] The conformational analysis derived from NMR data coupled with MD (Molecular Dynamic), Docking and CORCEMA-ST calculations has then provided the ligands bioactive conformation and the structural features of the complexes. Our results allowed to deeply characterize, at a molecular level, different systems of Siglec-2/sialoglycans complexes.[2,3]

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FUNCTIONALIZATION OF CHITOSAN-PGA BASED NANOPARTICLES FOR MULTIMODAL DIAGNOSIS IN TYPE I DIABETES REGENERATIVE THERAPY

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Diabetes is one of the most hard and challenging diseases for modern medicine research and iNanoBIT [1] project focus on new medical devices able to produce insulin for diabetic patients. In this context we plan to take advantage of nanotechnology using functionalized nanoparticles, obtained from poly(glycolic acid) (PGA), a biodegradable aliphatic polyester and Chitosan, a linear polysaccharide, BBS's aggregation protocol. Specifically, nanoparticles will be decorated with: a) recognition motifs of beta cells receptors; b) a chelator agent that allow PET and SPECT analysis, a NIR dye to develop a new optoacoustic diagnostic protocol (MSOT).

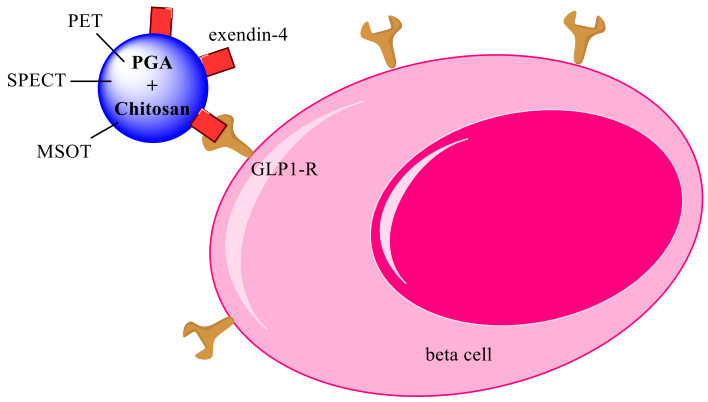

Figure 1: Nanoparticle and its identification on the beta-cell

Acknowledgments: this project is funded by **H2020-NMBP-15-2017- GA-760986 — iNanoBIT** (1.10.2017-30.9.2022) Integration of Nano- and Biotechnology for beta-cell and islet Transplantation. **http://inanobit.eu/about-inanobit/**

[1] inanobit.eu/about-inanobit/

SELECTIVE FUCOSE RECOGNITION IN WATER BY A BIOMIMETIC SYNTHETIC RECEPTOR.

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Carbohydrates are essential mediators of a wide range of both physiological and pathological processes which rely on the recognition of specific saccharidic units, mainly exerted by several classes of proteins such as lectins.[1] Mimicking lectin function in specific recognition events could affect carbohydrate-mediated processes opening the way to possible therapeutic applications.[2] Biomimetic synthetic receptors that recognize carbohydrates by the same non covalent interactions used by lectins in nature may represent powerful tools featuring useful advantages over their natural counterparts.[2] Although over the last decades a significant effort has been dedicated to the design of biomimetic receptors for carbohydrates,[3] at present effective recognition in water was achieved only toward glucose and related sugars.[4] Therefore, expanding the realm of biomimetic receptors to different classes of saccharides represents a target of paramount importance. In the last decade we have been involved in molecular recognition of carbohydrates, mostly developing diaminopyrrolic synthetic receptors recognizing saccharides of biological interest.[5] Despite effective recognition properties in competitive organic media, no evidence of binding could be detected in water, mainly because of the insolubility of the prepared structures. To overcome this problem we have designed a new receptor in which the diaminopyrrolic moiety has been replaced with a diaminocarbazolic unit featuring two phosphonate groups, in order to increase water solubility. In this communication, we present design, synthesis and binding properties of a water soluble receptor based on a diaminocarbazolic unit, which selectively recognizes fucose in water.[6]

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A NEW SET OF ORTHOGONAL PROTECTING GROUPS FOR A DISACCHARIDE SYNTHON LEADING TO CONJUGABLE LIPID A ANALOGUES

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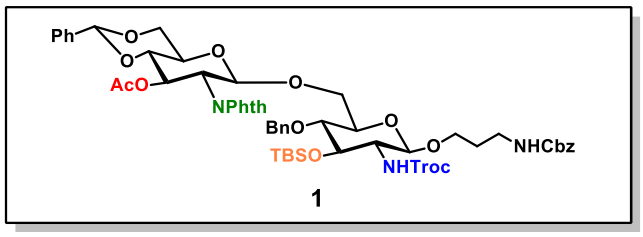
Generation of strong immune responses to poorly immunogenic antigens requires the help of an immunostimulatory adjuvant. This has led to recent resurgence in interest on the development of new adjuvants.[1] Administration of Lipopolysaccharide (LPS) or its biologically active component Lipid Ais known to cause systemic response through activation of Toll like receptor 4. These adjuvants are generally too toxic to be used as adjuvants for human vaccines.[2] Lipid A possesses a unique and archetypal structure characterized by amino sugar disaccharide backbone which in most cases comprises two 2-amino-2-deoxy-d-glucopyranose ($_D$-GlcpN) residues conjugated through a β (1–6) linkage to which typically two negatively charged phosphates at the 1 and 4′ positions are attached.[2,3]

The synthesis of well-defined Lipid A analogues has led to understand the structure-activity relationship of this class of molecules and develop vaccine adjuvants.[4] A synthetic TLR4 agonist has been also able to function as carrier for carbohydrate antigens.[5]

The aim of this work is to investigate a convergent synthesis of disaccharide **1** with a new set of orthogonal protecting groups leading to a series of Lipid A analogues with a linker for conjugation. This disaccharide is a key synthon to achieve the synthesis of a variety of potential adjuvants to boost the immune response of vaccine.



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IDENTIFICATION AND SYNTHESIS OF ANALYTICAL STANDARDS FOR QUANTIFICATION OF *N. MENINGITIDIS* CAPSULAR POLYSACCHARIDE SEROGROUP A CARBA ANALOGUES

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Neisseria meningitidis type A (MenA) is a Gram negative encapsulated bacterium that is a major cause of epidemic meningitis, particularly across an area known as the "African Meningitis Belt"[1]. The capsular polysaccharide (CPS) represents a major virulence factor of MenA and consists of (1→6)-linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units. Monovalent and multivalent vaccine based on CPS are available, and the incidence of infection is declining worldwilde and also in Africa.

The presence of phoshodiester renders the CPS structure hydrolitically unstable and hampers the development of liquid formulations. Conversion of natural sugars into carbocycles has been proposed as a strategy to increase the stability of MenA CPS[2]. Oligomers from one to three repeating unit have been synthesized and conjugated to the carrier protein CRM_{197}[3]. Only the trimer showed to be immunogenic in mice model although inferior to the vaccine bench mark [2,3]. For the characterization of these type of conjugates, the development of an analytical method to quantify the bound and unbound oligomer is important. To this end, work is ongoing to identify and synthesize analytical standard for MenA carba analogues quantification.

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SYNTHESIS OF AN AMINOACID CONTAINING ANALOGUE OF THE *STREPTOCOCCUS PNEUMONIAE* TYPE 14 (SP14) REPEATING UNIT

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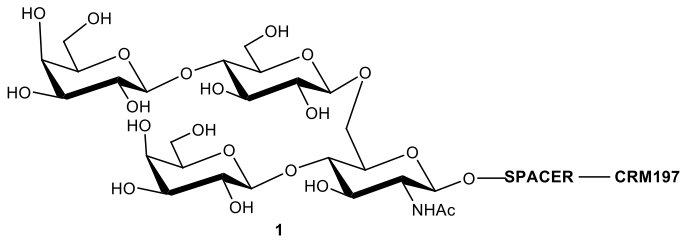
Many pathogenic bacteria have capsules constituted mainly by polysaccharides (CPS) which are able to induce the production of antibodies and give a specific antibacterial protection.[1] *Streptococcus pneumoniae* type 14 (SP14) is a serotype with high clinical relevance and recent studies showed that the tetrasaccharide repeating unit **1** represents the smallest structure of the CPS that is able to elicit antibody responses against SP14, when linked to an immunogenic protein.[2]

Various studies demonstrate that a few zwitterionic capsular polysaccharides (ZPS) can activate a T-dependent antibody response without the need of the protein conjugation. Based on these intriguing results, we are currently studying the potential of new zwitterionic structures. Herein we present our preliminary results in the preparation of tetrasaccharide **2**, which contains an amino acidic zwitterionic motif, as an analogue of the repeating unit **1**.



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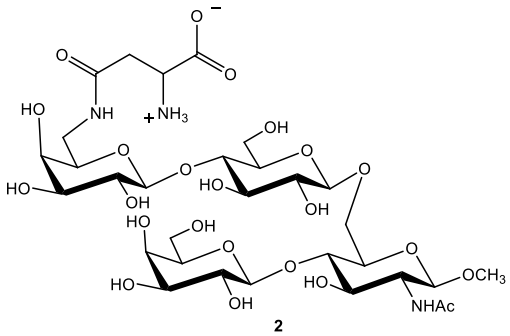

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The target structure has been built up by using lactose, glucosamine, and galactose as starting materials.

The newly synthesized SP14 CPS analogue (**2**) will be tested for its biocompatibility and immunological properties.

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Acknowledgements: This work has been supported by MIUR-Italy (PRIN 2015, Prot. 2015RNWJAM)

SYNTHESIS OF GLYCO-CONJUGATES OF 1,2-DIHYDRO-2-OXO-PYRIDINE-3-CARBOXAMIDES AS NEW MODULATORS OF ENDOCANNABINOID SYSTEM

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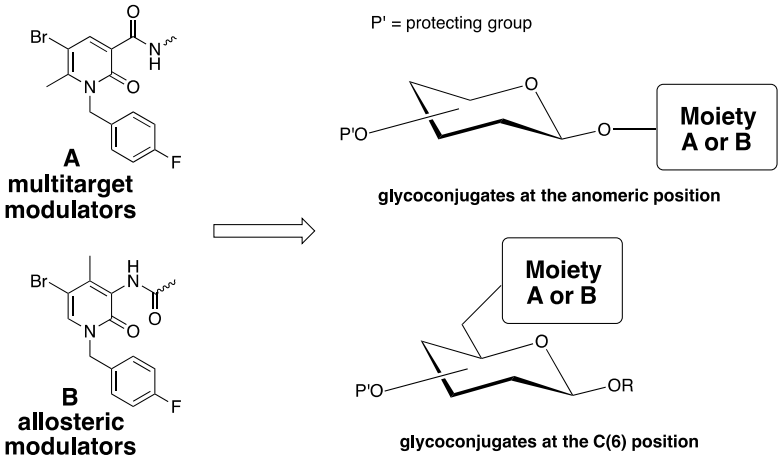
The endocannabinoid system (ECS) is a ubiquitous complex lipid signaling system, involved in different physiological and pathological processes. This system is composed by cannabinoid receptors (CB1R and CB2R), endocannabinoids (ECs) and the enzymes for EC synthesis and degradation (FAAH, MAGL, ABHD6 and ABHD12). Multi target compounds exert their pro-cannabinoid activities by acting via the simultaneous modulation of more targets and might represent a promising pharmacological modulation of the ECS. Moreover, another new strategy consists in the development of allosteric modulators of the cannabinoid receptors which might modulate the ECS with minimized side-effects and might boost beneficial effects of endogenously released endocannabinoids as well as synergize with other ECS modulators.

Previously we developed multi target compounds of ECS [1] and positive allosteric modulators of CB2R [2] of general structure **A** and **B** respectively.

Herein, we report our preliminary results in the synthesis of a series of new cannabinoid receptor ligands obtained from the conjugation of 1,2-dihydro-2-oxo-pyridine-3-carboxamides portion of **A** or **B** with the anomeric or C(6) position of monosaccharide units (D-Gluco, D-galacto and GLcNAc). The new compounds were designed with the aim to increase hydrophilicity and activity of multitarget compounds **A** and of CB2R positive modulators **B**.



The new compounds will be tested for their biocompatibility and biologically properties.

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Acknowledgment: This research was supported by grants from University of Pisa (Progetti di Ricerca di Ateneo, PRA_2017_51) and by Fism –Fondazione Italiana Sclerosi Multipla- cod 2017/R/16 and financed or co-financed with the "5 per mille" public funding.

STEREOSELECTIVE SYNTHESIS OF NEW IMINO ANALOGUES OF MINIMAL EPITOPE MANα(1,2)MAN AS POTENTIAL DC-SIGN LIGANDS

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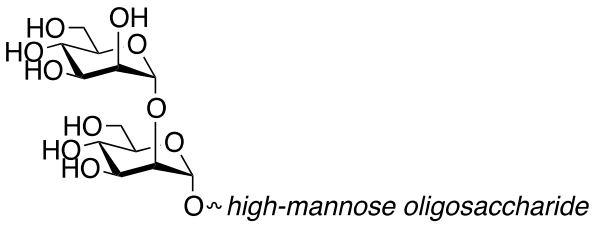
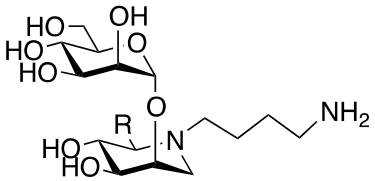
Dendritic cells (DC) are professional antigen presenting cells (APC) that are positioned throughout peripheral tissues to act as sentinels against invading pathogens.
DC-SIGN (Dendritic Cell-Specific ICAM-3 Grabbing Nonintegrin) is a tetrameric C-type lectin presenting four copies of a carbohydrate recognition domain (CRD) at the *C* terminus. This calcium dependent lectin specifically recognizes highly-glycosylated structures present at the surface of several pathogens.[1]
The main carbohydrate ligand recognized by DC-SIGN is the high mannose glycan $(Man)_9(GlcNAC)_2$, which is present on the surface of some pathogens (e.g. Gp120 in HIV) and the minimal epitope is represented by the terminal disaccharide portion Manα(1,2)Man, which plays a major role in DC-SIGN recognition and binding.[2]
Recently, in our laboratory new mimics of Manα(1,2)Man were synthesized. They are composed by two moieties: a mannose portion as the non-reducing unit, and the reducing moiety, where the mannose is replaced by a real D-carbamannose core, or by more lipophilic carba-analogues, which confer enzymatic stability to the molecule, and a chemical profile corresponding to that of Manα(1,2)Man. All these pseudo-1,2-α-mannobiosides showed a good DC-SIGN binding profile, comparable to that of the natural disaccharide.[3]
On the basis of these results that confirm the validity of the DC-SIGN antagonism by small molecules, we developed the stereoselective synthesis of the new iminopseudomannobiosides **1** and **2**, mimic of the natural Manα(1,2)Man, bringing a new iminomannose unit linked to the typical mannose portion. The crucial step in the formation of the imino-sugar portion is represented by the double reductive amination of the appropriate dicarbonyl-intermediate.[4]



minimal epitope Manα(1,2)Man



*iminopseudomannobiosides **1** and **2***

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Acknowledgment: This research was supported by grants from University of Pisa (Progetti di Ricerca di Ateneo, PRA_2017_51).

CHARACTERIZATION OF GLYCOSYLATED E. COLI L-ASPARAGINASE II BY NMR

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Bioconjugated enzymes are used in medicine and material science, but classical techniques cannot be used for their characterization. The functionalization prevents crystallization and X-ray analyses. Moreover the large molecular weight of bioconjugated proteins hinders the employment of solution NMR. Conversely, solid-state NMR (ssNMR) does not suffer from size limitations and it is effective for the study of systems which even lack of long-range three-dimensional order.

Several bioconjugation approaches have been applied to *E. coli* L-asparaginase II (ANSII) which is used as drug − mainly in its PEGylated form − for the treatment of acute lymphoblastic leukemia (ALL). Since its medical and favorable structural characteristics, we chose ANSII as a model to be differently biocojugated and characterized by ssNMR in order to develop a new efficient methodology for the study of this kind of systems. We already functionalized ANSII with PEG chains **[1]** and gold nanoparticles (AuNPs) **[2]**. Both systems were successfully characterized by ssNMR which proved the preservation of structural integrity after functionalization, showing the efficacy and reliability of this tool.

As further develop, ANSII has been functionalized with *Neisseria meningitidis* serogroup C (MenC) capsular polysaccharide **[3]** to simulate a carbohydrate-based vaccine which is the safest and most effective kind of vaccines for the prevention of bacterial infectious diseases. Even in this case, ssNMR has been used for the structural investigation and it has turned out to be efficient not only for the evaluation of structural preservation after glycosylation but also for a more detailed evaluation of the conjugation pattern.

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ATOMIC STRUCTURAL DETAILS OF A PROTEIN GRAFTED ONTO GOLD NANOPARTICLES

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The development of a methodology for the structural characterization at atomic detail of proteins conjugated to nanoparticles would be a breakthrough in nanotechnology. Solution and solid-state NMR spectroscopies are currently used to investigate molecules and peptides grafted onto nanoparticles, but the strategies used so far fall short in the application to proteins, which represent a thrilling development in theranostics. We have demonstrated the feasibility of highly-resolved multidimensional heteronuclear spectra of a large protein assembly conjugated to PEGylated gold nanoparticles. The spectra have been obtained by direct proton detection under fast MAS and allow for both a fast fingerprinting for the assessment of the preservation of the native fold and for resonance assignment. We thus demonstrate that the structural characterization and the application of the structure-based methodologies to proteins bound to gold nanoparticles is feasible and potentially extensible to other hybrid protein-nanomaterials.

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