Selection of the biological activity of DNJ neoglycoconjugates through click length variation of the side chain

Nicolas Ardes-Guisot, a Dominic S. Alonzi, b Gabriele Reinkensmeier, b Terry D. Butters, b Caroline Norez, c Frédéric Becq, c Yosuke Shimada, d Shinpei Nakagawa, d Atsushi Kato, d Yves Blériot,* e/f Matthieu Sollogoub, c and Boris Vauzeilles* a

a CNRS, Glycochimie Moléculaire et Macromoléculaire, ICMMO, UMR 8182, Orsay F-91405, France. Fax: +33 1 69 15 47 15; Tel: +33 1 69 15 68 36; E-mail: boris.vauzeilles@u-psud.fr; Univ Paris Sud, Orsay, F91405

b Glycobiology Institute, Oxford University, South Parks Road, Oxford OX1 3QU, UK. Fax: +44 1 865 275 21671

c Institut de Physiologie et Biologie Cellulaires, Université de Poitiers, CNRS, 1 rue Georges Bonnet, BP633, 86022 Poitiers cedex, France.

d Department of Hospital Pharmacy, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.

 e UPMC, Univ Paris 06, Institut Parisien de Chimie Moléculaire, (UMR CNRS 7201), 4 place Jussieu, C. 181, F-75005 Paris, France. Fax: + 33 1 44 27 55 04

f Present address : Université de Poitiers, Laboratoire de Synthèse et Réactivité des Substances Naturelles, UMR CNRS 6514, 4 avenue Michel Brunet, F-86022 Poitiers, France.
Synthesis of functionalised linkers

A series of linkers displaying different combinations of functionalities were synthesized. Starting from commercially available alcohols 20b and 20c, 4 and 6 carbon spacers 6b and 6c bearing a terminal alkyne on one end and a terminal tosylate on the other end were efficiently prepared through standard tosylation. The synthesis of tethers displaying a terminal azide was also achieved, in which commercial halogenoalcohols 21a-d of various length were displaced with sodium azide to generate the azidoalcohols 22a-d. Several aprotic solvents (acetone, acetonitrile, DMF) were screened for this reaction and DMF gave the best results. Subsequent tosylation furnished the tosyl azides 5a-d in good yield.

\[
\begin{align*}
&20b \quad n = 2 \\
&20c \quad n = 4 \\
&6b \quad n = 2 (98\%) \\
&6c \quad n = 4 (81\%) \\
&21a \quad n = 4, R=Ac, X=Cl \\
&21b \quad n = 6, R=H, X=Br \\
&21c \quad n = 8, R=H, X=Br \\
&21d \quad n = 10, R=H, X=Br \\
&22a \quad n = 4 (74\%) \\
&22b \quad n = 6 (64\%) \\
&22c \quad n = 8 (97\%) \\
&22d \quad n = 10 (64\%) \\
&5a \quad n = 4 (86\%) \\
&5b \quad n = 6 (86\%) \\
&5c \quad n = 8 (91\%) \\
&5d \quad n = 10 (83\%)
\end{align*}
\]

Scheme S1. Reagents and conditions: a) TsCl, Et₃N, CH₂Cl₂, 0°C to RT; b) NaN₃, DMF, 80°C, 24 h; followed by K₂CO₃, CH₃OH, RT, 18 h for 5a only.

Synthesis of the adamantane precursors

Two adamantane precursors bearing either an O-propargyl moiety or a primary azide were used. Adamantanemethanol 9 was reacted with propargyl bromide to yield the propargylic derivative 10 (58% yield). Tosylation of alcohol 9 followed by azide displacement gave the azidomethyl adamantane 11 in 74% yield over 2 steps.

\[
\begin{align*}
&10 \\
&9 \\
&11
\end{align*}
\]

Scheme S2. Reagents and conditions: a) NaH, propargyl bromide, DMF, 0°C to RT, 18 h, 58%; b) TsCl, Et₃N, CH₂Cl₂, 0°C to RT then NaN₃, DMF, 80°C, 74% over two steps.

N-alkylation of DNJ

Planning to apply in the forthcoming step the click glycosylation conditions we have developed for unprotected sugars, we first attempted the direct N-alkylation of unprotected DNJ 1 thus avoiding protecting groups manipulation. N-Alkylation through reductive amination with linear linkers bearing a terminal aldehyde was first studied but afforded the desired N-alkyl DNJ derivatives only in modest yield, especially with longer chains. Disappointing results were also obtained when switching to the N-alkylation of DNJ with alkyl tosylates or bromides. These results prompted us to abandon this route and concentrate instead on the N-functionalisation of tetra-O-benzyl-deoxynojirimycin 4. N-Alkylation of 4 with alkyl chains bearing an alkyne (propargyl bromide 6a and 6b-6c) or an azide moiety (5a-
5d) was examined and reaction parameters including temperature, solvent (acetonitrile, DMF) and base (NaH, Na$_2$CO$_3$, K$_2$CO$_3$) were screened (Table S1). Heating to 90°C was necessary for a good conversion and potassium carbonate proved to be the best base to perform the N-alkylation either starting from alkyl bromides or alkyl tosylates. However, since the preparation of bromides required one more synthetic step, the combination of alkyl tosylate and potassium carbonate was chosen as optimal conditions in which acetonitrile gave better results than DMF. In the case of the azido-containing chains 5a-5d, chain length governed the yield for N-alkylation, lower conversion being observed for longer chains. This can be tentatively explained by the increased lipophilicity of the chain compared to the relative hydrophilicity of the DNJ derivative 4.

![Scheme S3](image)

<table>
<thead>
<tr>
<th>$N$-alkyl</th>
<th>X</th>
<th>Base</th>
<th>Solvent</th>
<th>T ($°C$)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7a</td>
<td>OTs</td>
<td>NaHCO$_3$</td>
<td>DMF</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>7a</td>
<td>Br</td>
<td>K$_2$CO$_3$</td>
<td>CH$_3$CN</td>
<td>Reflux</td>
</tr>
<tr>
<td>4</td>
<td>7a</td>
<td>OTs</td>
<td>K$_2$CO$_3$</td>
<td>CH$_3$CN</td>
<td>Reflux</td>
</tr>
<tr>
<td>6</td>
<td>7b</td>
<td>OTs</td>
<td>NaHCO$_3$</td>
<td>DMF</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>7b</td>
<td>Br</td>
<td>K$_2$CO$_3$</td>
<td>CH$_3$CN</td>
<td>Reflux</td>
</tr>
<tr>
<td>6</td>
<td>7b</td>
<td>OTs</td>
<td>K$_2$CO$_3$</td>
<td>CH$_3$CN</td>
<td>Reflux</td>
</tr>
<tr>
<td>8</td>
<td>7c</td>
<td>OTs</td>
<td>NaHCO$_3$</td>
<td>DMF</td>
<td>90</td>
</tr>
<tr>
<td>8</td>
<td>7c</td>
<td>Br</td>
<td>K$_2$CO$_3$</td>
<td>CH$_3$CN</td>
<td>Reflux</td>
</tr>
<tr>
<td>8</td>
<td>7c</td>
<td>OTs</td>
<td>K$_2$CO$_3$</td>
<td>CH$_3$CN</td>
<td>Reflux</td>
</tr>
<tr>
<td>10</td>
<td>7d</td>
<td>OTs</td>
<td>NaHCO$_3$</td>
<td>DMF</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>7d</td>
<td>Br</td>
<td>K$_2$CO$_3$</td>
<td>CH$_3$CN</td>
<td>Reflux</td>
</tr>
</tbody>
</table>

Table S1 Yields and conditions for the alkylation of 4 with azido linkers

A similar study was conducted with alkyne derivatives (Table S2) showing that the use of potassium carbonate in refluxing acetonitrile gave slightly better yields than sodium hydrogenocarbonate in DMF (90°C).

![Scheme S4](image)

Scheme S4.
<table>
<thead>
<tr>
<th>n</th>
<th>Product</th>
<th>X</th>
<th>Base</th>
<th>Solvent</th>
<th>T (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8a</td>
<td>Br</td>
<td>NaHCO₃</td>
<td>DMF</td>
<td>90</td>
<td>89</td>
</tr>
<tr>
<td>1</td>
<td>8a</td>
<td>Br</td>
<td>K₂CO₃</td>
<td>CH₃CN</td>
<td>Reflux</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>8b</td>
<td>OTs</td>
<td>NaHCO₃</td>
<td>DMF</td>
<td>90</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>8b</td>
<td>OTs</td>
<td>K₂CO₃</td>
<td>CH₃CN</td>
<td>Reflux</td>
<td>71</td>
</tr>
<tr>
<td>4</td>
<td>8c</td>
<td>OTs</td>
<td>NaHCO₃</td>
<td>DMF</td>
<td>90</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>8c</td>
<td>OTs</td>
<td>K₂CO₃</td>
<td>CH₃CN</td>
<td>Reflux</td>
<td>74</td>
</tr>
</tbody>
</table>

Table S2 Yields and conditions for the alkylation of 4 with alkyne linkers

**Click coupling**

Copper-catalyzed Azide-Alkyne cycloaddition (CuAAC)\(^{11}\) between the N-alkylated DNJ derivatives 7a-d and 8a-c and the adamantane partners 10 and 11 was then examined. Optimised click reaction conditions that were previously established in our laboratory regarding temperature (room temperature), substrate concentration (0.25M), the amounts of copper sulfate (5%) and sodium ascorbate (15 mol%)\(^{7}\) were used in a dichloromethane/water (1:1) solvent mixture which proved better than the extensively used aqueous alcohol systems (tables S3 and S4).\(^{12}\) Noteworthily, we observed that reaction in a screw-cap sealed tube allowed optimum conversion as undesired reoxidation of copper I to copper II was otherwise probably taking place during the reaction. These conditions were applied to the 1,3-cycloaddition of all complementary adamantane and DNJ partners.

![Scheme S5](image)

Table S3 Yields and conditions for the cycloaddition of 8a-c with 11.
Scheme S6.

<table>
<thead>
<tr>
<th>n</th>
<th>Product</th>
<th>Screw-capped Tube</th>
<th>Solvent</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>12a</td>
<td>No</td>
<td>CH$_2$Cl$_2$/H$_2$O</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>12a</td>
<td>Yes</td>
<td>CH$_2$Cl$_2$/H$_2$O</td>
<td>86</td>
</tr>
<tr>
<td>6</td>
<td>12b</td>
<td>No</td>
<td>CH$_2$Cl$_2$/H$_2$O</td>
<td>64</td>
</tr>
<tr>
<td>6</td>
<td>12b</td>
<td>Yes</td>
<td>CH$_2$Cl$_2$/H$_2$O</td>
<td>93</td>
</tr>
<tr>
<td>8</td>
<td>12c</td>
<td>No</td>
<td>CH$_2$Cl$_2$/H$_2$O</td>
<td>77</td>
</tr>
<tr>
<td>8</td>
<td>12c</td>
<td>Yes</td>
<td>CH$_2$Cl$_2$/H$_2$O</td>
<td>97</td>
</tr>
<tr>
<td>10</td>
<td>12d</td>
<td>No</td>
<td>CH$_2$Cl$_2$/H$_2$O</td>
<td>62</td>
</tr>
<tr>
<td>10</td>
<td>12d</td>
<td>Yes</td>
<td>CH$_2$Cl$_2$/H$_2$O</td>
<td>96</td>
</tr>
</tbody>
</table>

Table S4: Yields and conditions for the cycloaddition of 7a-d with 10.
Figure S1. NP-HPLC analysis of FOS in HL60 cells following inhibitor treatment. Compounds were incubated for 24h with cells, FOS extracted, fluorescently labelled and separated by NP-HPLC as described in the text. A, representative profile for our compounds showing the Glc$_3$Man$_5$GlcNAc$_1$ species that was measured in response to high concentrations of inhibitor effecting glucosidase I activity; B, profile of our compounds following low concentration treatment (5 $\mu$M) showing major species Glc$_1$Man$_4$GlcNAc$_1$ as a result of measured glucosidase II inhibition. Data for peak area were used to construct Table 2 in the paper.

Figure S2. NP-HPLC analysis of GSL in HL60 cells following inhibitor treatment. Compounds were incubated for 3 days with cells, GSL extracted, ceramide glycanase treated and the released oligosaccharide fluorescently labelled and separated by NP-HPLC as described in the text. Representative profile showing the reduction in the major species,
ganglioside GM3, following treatment with our compounds. Data for peak area were used to construct Figure 4 in the paper.

**Cytotoxicity assay**

HL60 cells were seeded at densities of 500 cells / well in 96-well plates in 200 μl of supplemented media containing 0.01 % DMSO as control, and concentrations up to 200 μM of each compound (dissolved in DMSO) added for 3 days. Cell viability was assessed in triplicate using the Cell Titer-96 AQueous cellular proliferation assay kit according to manufacturer’s (Promega, Southampton, UK) instructions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP-DNJ</td>
<td>&gt;200</td>
</tr>
<tr>
<td>NB-DNJ</td>
<td>&gt;200</td>
</tr>
<tr>
<td>14</td>
<td>&gt;200</td>
</tr>
<tr>
<td>15</td>
<td>&gt;200</td>
</tr>
<tr>
<td>16</td>
<td>&gt;200</td>
</tr>
<tr>
<td>17</td>
<td>&gt;200</td>
</tr>
<tr>
<td>18</td>
<td>&gt;200</td>
</tr>
<tr>
<td>19</td>
<td>65 ± 8</td>
</tr>
<tr>
<td>20</td>
<td>19 ± 3</td>
</tr>
</tbody>
</table>

**Table S5.** Cytotoxicity of compounds in HL60 cells over a 3-day incubation time.

![Figure S3. Representative Hill Slope plot of β-glucocerebrosidase inhibition.](image)
Figure S4. Functional evaluation of F508del-CFTR by DiSBAC2(3) assay in CFKM4 cells treated with 100μM of compounds during 2h. Examples of typical time courses obtained with untreated or 14-treated cells. Data represent the mean (± SEM) of the relative fluorescence collected from 12 cells of a field. Mixture of forskolin (10 μM) + genistein (30 μM) is used to activate CFTR. CFTRinh-172 (10 μM) is used to inhibit CFTR.

Figure S5. F508del-CFTR activity was assayed with iodide efflux technique in presence of forskolin (10 μM) + genistein (30 μM). Examples of iodide efflux curves as function of time on CF-KM4 cells treated or not by 14 (100 μM, 2h). Results are expressed as mean ± SEM of 4 experiments.

References and notes

In the case of the four carbon chain 5a, a slightly more elaborate route, involving protection of the hydroxyl as an acetate (22a), followed by deprotection after the introduction the azido group, proved necessary due to competing THF formation when nucleophilic substitution was attempted directly from 4-chloro-butan-1-ol.


These aldehydes were obtained by straightforward PCC oxidation of the corresponding alcohols and in refluxing dichloromethane.


Bromides were obtained by nucleophilic displacement of tosylates 6a-b and 9a-d using LiBr in acetone.


List of NMR characterisations,

Compound 7a, $^1$H, $^{13}$C, COSY, HSQC
Compound 7b, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 7c, $^1$H, $^{13}$C, COSY, DEPT, HSQC
Compound 7d, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 8a, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 8b, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 8c, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 12a, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 12b, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 12c, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 12d, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 13a, $^1$H, $^{13}$C, COSY, HSQC, HMBC
Compound 13b, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 13c, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 14, $^1$H, $^{13}$C, COSY, HSQC
Compound 15, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 16, $^1$H, $^{13}$C, COSY, DEPT, HSQC
Compound 17, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 18, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 19, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Compound 20, $^1$H, $^{13}$C, COSY, DEPT, HSQC, HMBC
Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry
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