Multifunctional multivalency: a focused library of polymeric cholera toxin antagonists

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ELISA Assay for Inhibition of Heat-labile Toxin (LT)

To PVC microtiter plates (Gibco BRL Inc.) solution of coating compound 9 (1 μg/mL, 100 μL/well) in PBS buffer was added and incubated at room temperature overnight, then washed (6×) with PBST (0.05 % Tween 20 in phosphate buffer saline, PBS). An inhibitor at decreasing concentrations (starting at 20 mg/mL, dilution factor 3.16; 50 μL/well) was mixed with LT (Sigma, 2 ng/mL) and the mixtures were applied to the plates. After incubation at room temperature for 2 h the plates were washed (6×) with PBST. Rabbit anti-LT polyclonal antibody (Abcam Inc., dilution 1:10000, 100 μL/well) was added, incubated at room temperature for 1 h then the plates were washed (6×) with PBST. Goat anti-rabbit IgG conjugated to horseradish peroxidase (Kirkegaard and Perry Laboratories, dilution 1:5000, 100 μL/well) was added, incubated at room temperature for 30 min then the plates were washed (6×) with PBST. 3,3,5,5-Tetramethylbenzidine solution (TMB purchased from KPL, 100 μL/well) was added. After 15 min incubation the reaction was stopped by addition of H3PO4 (100 μL/well) and absorbance was measured at 450 nm.

Table 1S. Inhibitory activities of heterobifunctional polymers against LT.

| Alkyne derivative 7.n | α-Gal polymer | β-Gal polymer | IC50, μM
|-----------------------|---------------|---------------|----------
| 5α27 (3%)             | 0.00099       | 5β27 (3%)     | 0.0011   |
|                       | (h=0.56)      |               | (h=0.8)  |
| 5α27 (5%)             | 0.00007       | 5β27 (5%)     | 0.0004   |
|                       | (h=0.54)      |               | (h=0.95) |
| 5α27 (9%)             | 0.00015       | 5β27 (9%)     | 0.00023  |
|                       | (h=1.04)      |               | (h=1.3)  |
| 6α27 (6.6%)           | 0.0006        | 6β27 (6.6%)   | 0.0017   |
|                       | (h=0.76)      |               | (h=1.0)  |
| 5α28 (5%)             | 0.0029        | 5β28 (5%)     | 0.0034   |
|                       | (h=1.3)       |               | (h=1.1)  |
| 5α28 (9%)             | 0.00007       | 5β28 (9%)     | 0.00013  |
|                       | (h=1.3)       |               | (h=1.4)  |
| 6α28 (6.6%)           | 0.00095       | 6β28 (6.6%)   | 0.001    |
|                       | (h=0.8)       |               | (h=0.74) |
| 6α28 (15%)            | 0.00019       | 6β28 (15%)    | 0.00084  |
|                       | (h=0.7)       |               | (h=0.7)  |

*a activities per pendant ligand. b Hill coefficient
Selected $^1$H, $^{13}$C NMR and IR spectra
4β
Dextran

Aminated dextran
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