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Synthesis and Cholera Toxin Binding Properties of a Lactose-2-aminothiazoline Conjugate

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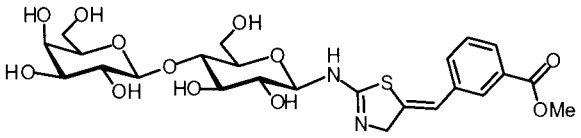
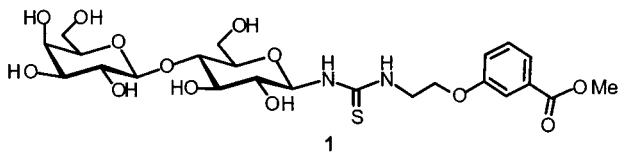
ABSTRACT



During the search for improved monovalent ligands for cholera toxin (CT), a new lactose-2-aminothiazoline conjugate was discovered. In a fluorescence binding assay the compound was found to be one of the strongest relatively simple CT ligands to date with a K_d of 23 μM.

Interference with protein−carbohydrate interactions has great medicinal potential in a variety of areas,[1] including the fight against bacterial infections[2] and toxins.[3] We are involved in a program to design interfering compounds in both monovalent and multivalent form. Cholera toxin (CT), the disease-causing agent produced by the pathogen *Vibrio cholerae*, is one of our target proteins. Inhibiting CT binding to its natural ligand GM1 in the intestinal tract should prevent the onset of the cholera disease.[3] As part of these studies we recently reported a relatively potent monovalent inhibitor for cholera toxin.[4] For this compound, lactose derivative **1**, we determined a K_d of 248 μM using a direct fluorescence binding assay. Both the thiourea moiety and the aryl group seemed to contribute to the enhanced binding as compared to that of unsubstituted lactose (K_d of 18 mM). To make further improvements we decided to synthesize other lactose derivatives, such as **2**, which contains a more rigid spacer between the sugar and aryl group yet includes a methylene unit permitting conformational adjustments.

The projected synthesis of **2** (Scheme 1) started by treatment of iodide **3** with Boc-protected propargylamine under modified Sonogashira reaction conditions.[5] Compound **4** was obtained in 62% yield after column chromatography (hexane/EtOAc, 80:20, v/v). The ^{13}C NMR spectrum of **4** showed the characteristic signals at δ 86 and 82 ppm for the carbons of the triple bond. The amine resulting from Boc removal of **5** using a CH_2Cl_2/TFA (5:2) mixture was treated with acetylated lactose β-isothiocyanate and iPr$_2$NEt in CH_2Cl_2. After the mixture stirred overnight, two new products were observed by TLC.[6] On attempted isolation of both of them by preparative TLC only a single compound was obtained. Apparently the higher running compound was converted to the lower running one on the silica gel plate. This process could also be induced by adding acetic acid to a dichloromethane solution of the mixture. Complete conversion to the lower running spot was observed within 3 h. The



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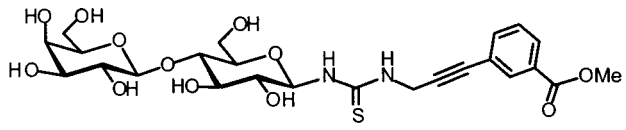

2

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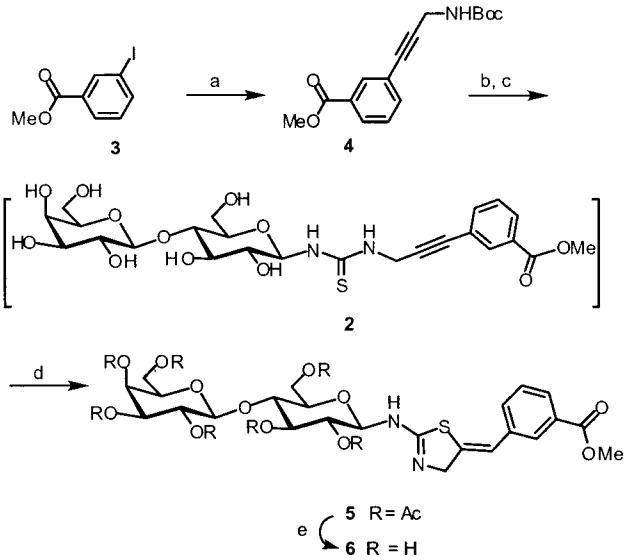
Scheme 1[a]



[a] (a) HC≡CCH$_2$NHBoc, Pd^0, CuI, NEt$_3$, CH$_3$CN, 14 h (62%); (b) TFA, CH$_2$Cl$_2$ (quant); (c) Lac(OAc)$_7$NCS, CH$_2$Cl$_2$, *i*Pr$_2$NEt, 14 h; (d) AcOH, CH$_2$Cl$_2$ (84% for 2 steps); (e) dioxane/MeOH/4 N NaOH, 15:4:2, (45%).

^{13}C NMR spectrum of this compound showed neither a peak at 184 ppm characteristic for the carbon of a thiourea moiety nor the expected triple bond signals. The observed spectral features were consistent with structure **5** (or its tautomer with the other nitrogen protonated). The structure assignment is partly based on the reported[7,8] reactions of propargylamines with isothiocyanates and the related reactions of propargylic isothiocyanates with amines and was confirmed by several 2D-NMR techniques (see Supporting Information for detailed structural characterization).

The carbohydrate moiety of **5** was deprotected with base to yield **6**. This compound is insoluble in water, but the compound obtained after treatment with dilute HCl or formic acid and lyophylization had good water solubility. Presumably both nitrogens were protonated at this stage, since it is expected that the pK_a should exceed 6.[9] A similar NMR analysis as was used for **5** confirmed the structure of **6**. A stability test for **6** of 1 week in aqueous solution showed the compound to be stable.

The affinity of **6** for the cholera toxin B subunit (CTB) was determined with a direct fluorescence binding assay.[4] CTB contains one tryptophan residue (Trp88), the fluorescence of which is affected by the binding of carbohydrate ligands. Upon addition of **6**, the decrease of the CTB fluorescence was practically complete at saturation. This phenomenon was also observed for **1**[4] and is in contrast to lactose, which induces only a minor fluorescence quenching. For **6** the fluorescence at 350 nm fitted well to a simple one-binding-site model and the K_d value obtained was 23 μM. This indicates that **6** binds 1 order of magnitude better than **1** and 780-fold better than lactose.[4] In comparison to other monovalent CT ligands **6** performs remarkably well. Exceptional remains the close GM1 mimic of Bernardi et al.[10] with a K_d value similar to GM1 itself (50 nM[11]), but their simplified analogue has a K_d of 190 μM.[12] For the closely related heat-labile enterotoxin of *E. coli*, Hol et al. have found a lead compound from screening a small library, *m*-nitrophenyl-α-D-galactopyranoside, that has a K_d value determined by ITC of 175 μM, while their best designed derivative binds with a K_d of 12 μM.[13]

In summary, the synthesis and characterization of a lactose-2-aminothiazoline conjugate **6** was described. This is a stable compound with no tendency to convert to the corresponding 2-aminothiazole under ambient conditions. The class of 2-aminothiazolines is of interest for a variety of biological activities,[14] and the synthetic method described here will be further explored in this context. Compound **6** proved to be a high-affinity monovalent CTB ligand of relatively simple structure and may contribute to the development of an effective cholera prophylactic. Computational studies to decipher the origin of its affinity along with work toward further affinity enhancement for effective competition with GM1 through multivalency are currently in progress.

Acknowledgment. The Royal Netherlands Academy of Arts and Sciences (KNAW) is gratefully acknowledged for support.

Supporting Information Available: Experimental procedures for the synthesis of **5** and **6** and their structure assignment including HMBC, HSQC, ROESY, and MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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