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Thiol–Disulfide Exchange Yields Multivalent Dendrimers of Melamine

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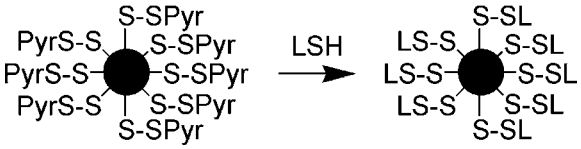
ABSTRACT



Thiol–disulfide exchange can be used to prepare multivalent conjugates of a small molecule or octapeptide displayed on dendrimers based on melamine. Exchange of four or eight thiopyridyl groups by captopril occurs at room temperature in methanol almost quantitatively. Exchange using the peptide requires higher temperatures and guanidinium chloride in DMF. While exchange on the tetravalent scaffold with four peptides is almost quantitative, sterics retard formation of the octavalent conjugate: the hexavalent conjugate forms readily.

As the products of multistep covalent syntheses, dendrimers are theoretically monodisperse macromolecules that can be designed to display specific functionality at the core and periphery.[1] This tailoring and the inherent potential for multivalency suggests the potential for dendrimers to be used as scaffolds for drug delivery.[2] Multivalency can increase the local concentration of ligands at a binding or target site and allow for more than one binding event.[3] The large molecular size and globular shape of dendrimers might facilitate accumulation in tumors, a phenomenon referred to as the enhanced permeability and retention effect (EPR)[4] or can provide a spool around which macromolecules such as DNA can wind.[5] Conjugates of dendrimers with drugs,[6] peptides,[7] carbohydrates,[8] and nucleic acids[9] have been developed to exploit multivalency and/or to address the need for better drug delivery systems. In contrast to these covalent strategies, using dendrimers as unimolecular micelles[10] is also being pursued, most notably, by Meijer with his dendritic box.[11]

Common to all of these studies is the significant burden of synthesis. To this end, a multivalent dendrimer scaffold that is amenable to post-synthetic manipulation to afford small libraries of molecules would be of great utility for evaluating structure−property relationships for a single binding phenomenon. We have shown that dendrimers based on melamine are tractable and can be engineered to display

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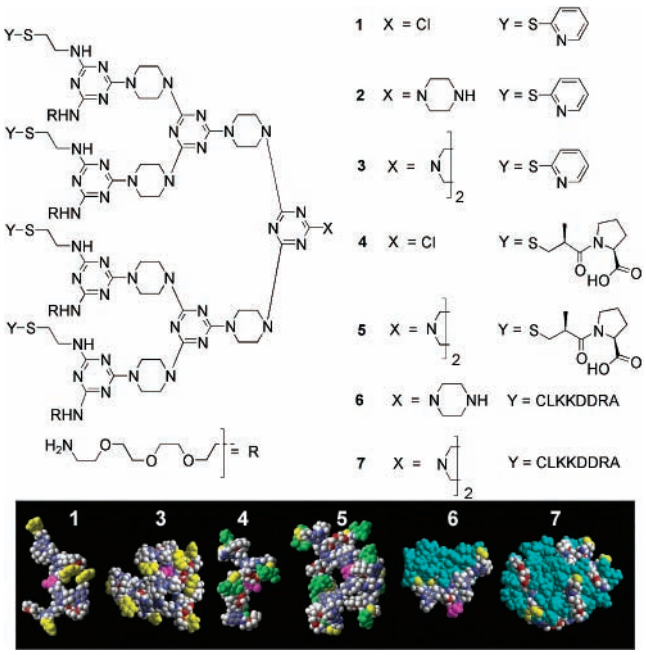
sub-stoichiometric numbers of reactive groups.[12] This level of structural diversity is a result of the differential reactivity of the triazine ring toward nucleophiles. Here, we describe water-soluble dendrimers based on melamine that display four or eight pyridyl disulfide groups (Scheme 1) that

Scheme 1. Target Molecules and Models[a]

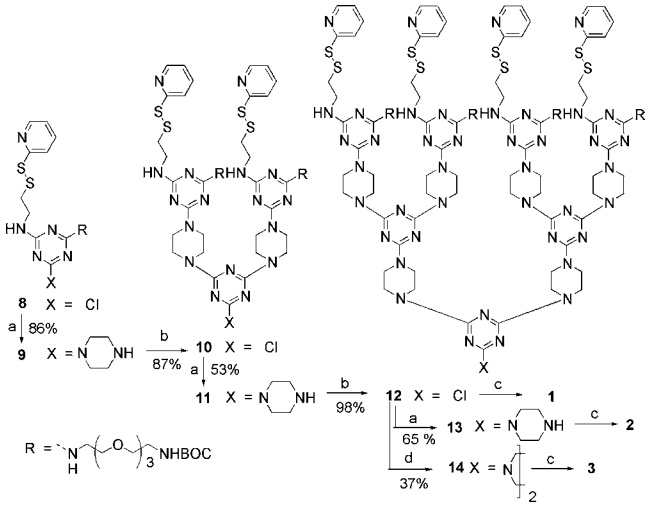


[a] Key: thiopyridyl groups (yellow), captopril (green), Peptides (blue), core (maroon).

undergo facile and irreversible thiol−disulfide exchange reactions with thiol-bearing ligands. To explore this chemistry with the eventual goal of preparing libraries of dendrimers, we report methods for the attachment of both a thiol-containing small molecule and a cysteine-containing peptide. The conjugates can be characterized by conventional methods including ^{1}H, ^{13}C spectroscopy and mass spectrometry.

The tetravalent dendrimers **1** and **2** and octavalent dendrimer **3** were synthesized convergently in six or eight steps in 26%, 17%, and 10% overall yields, respectively using a common synthetic pathway (Scheme 2). All intermediates were purified by flash column chromatography and gave satisfactory ^{1}H and ^{13}C spectra[13] and single parent ions by mass spectrometry.[14] The deprotection steps to provide **1**−**3** were judged to be quantitative by the absence of any other species identifiable in the NMR or mass spectra following purification by precipitation from diethyl ether. HPLC chromatograms (vida infra) corroborate purity.

Scheme 2. Syntheses of **1**−**3**[a]



[a] Key: (a) piperazine (excess), DIPEA, rt, 12 h; (b) $C_3N_3Cl_3$ (0.5 equiv), DIPEA, rt, 72 h; (c) 1:1 TFA/CH_2Cl_2; (d) piperazine (0.5 equiv), DIPEA, 50 °C, 48 h.

Functionalization of the tetravalent scaffolds through thiol−disulfide exchange between **1** and captopril gave conjugate **4** in almost quantitative yield under mild conditions; room temperature in CH_3OH in 5 h. The course of the reaction can be followed either spectrophotometrically with the appearance thiopyridinone or using HPLC (Figure 1a). The product can be isolated from the reaction mixture by precipitation with diethyl ether.

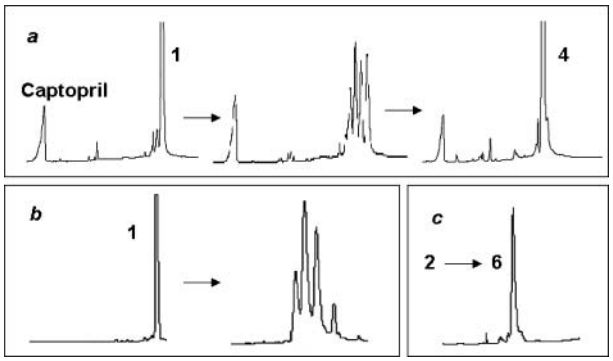


Figure 1. (a) Exchange on **1** with captopril yields **4** (t = 0, 40 min, 5 h). (b) Exchange on **1** with peptide (t = 48 h). See text for details. (c) Exchange on **2** yielding **6** (t = 3 h). Each trace is 20 min wide commencing with 10 min.

NMR characterization of **4** by total correlation spectroscopy (TOCSY) revealed chemical shift differences for both

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(13) The ^{1}H NMR spectra between δ 2.4 and 4.0 ppm is indicative of the stage of the iterative syntheses: piperazine protons proximate to a triazine and free secondary amine, respectively are unique. The ^{13}C NMR spectra of monochlorotriazines show a peak around 169 ppm, corresponding to the triazine carbon bearing the chlorine. While this peak is absent in piperazine derivatives, the peaks at δ 46 and 44 are present corresponding to the piperazine carbons.

(14) ESI-TOF and/or MALDI-TOF spectra of the intermediates and the dendrimers show fragmentation of the pyridyl disulfide groups (−110 amu) and corroborate the identity of the intermediates and products. Alkyl disulfides that result from exchange are much less labile: lines corresponding to loss of ligand (or incomplete reaction) are neglible.

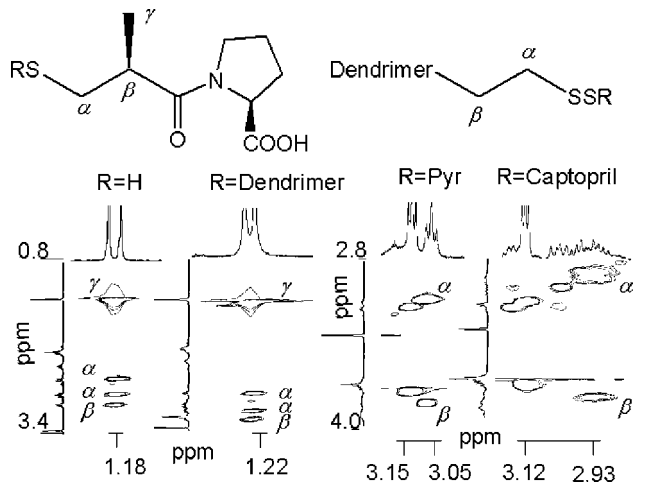

Figure 2. 2-D ^{1}H NMR spectra of captopril and the dendrimer reveal differences in chemical shift upon exchange.

the captopril group and the dendrimer upon exchange (Figure 2). Captopril protons shift downfield upon disulfide formation. The methylenic protons of the dendrimer shift upfield *and* become diastereotopic as a result of the chiral captopril group. The ^{13}C NMR spectra reveal disulfide exchange. As expected, the carbon adjacent to the thiol of captopril shifts from 28 ppm before exchange to 42 ppm upon oxidation.[15] MALDI-TOF of **4** confirms the identity of the tetrasubstituted product showing a single parent ion for the products.

Exchange between **1** and the peptide is significantly slower than the reaction with captopril: in CH_3OH exchange is not complete after 24 h (Figure 1b) but shows the mono- through tetrasubstituted products. At 80 °C in DMF in the presence of guanidinium chloride, the HPLC chromatograms show that the reaction is complete in 3 h (Figure 1c). While a single species is observed by HPLC, MALDI-TOF MS reveals a mixture of two tetravalent scaffolds, the desired monochlorotriazine and a species with a molecular weight consistent with substitution of the chlorotriazine with thiopyridine. By substituting the monochlorotriazine with piperazine, yielding **2**, this problem is circumvented and the exchange reactions of **2** proceed cleanly to afford a single product **6.** The proton NMR is crowded in the region of conjugation but clearly shows the downfield shift of 0.2 ppm is observed for the methylenic protons of cysteine.

Functionalization of **3** with captopril and CLKKDDRA is more challenging. Unfortunately, reactions with **3** cannot be monitored with HPLC: the size and polarity of the dendrimer precludes resolution of the starting material from any of the intermediates obtained on exchange. We adopted the strategy of a 48 h incubation time, consistent with appearance of thiopyridinone. Functionalization of dendrimer **3** with captopril yields a single product, **5**, that is easily isolated by precipitation. Both NMR spectroscopy and MALDI-TOF mass spectrometry corroborate assignment.

Exchange to yield the octavalent peptide conjugate is problematic. The reaction proceeds quickly to the hexavalent product in 2 days. The octavalent scaffold is not observed by mass spectrometry even in the presence of an excess of peptide for periods of 10 days, an issue that we attribute to steric congestion around the site of exchange. Consistent with this hypothesis, the observed hexavalent scaffold contains two exchangeable thiopyridyl groups as judged from MALDI-TOF MS. Computational models are consistent with steric congestion (Scheme 1). Captopril groups are sufficiently small that they do not preclude approach of an additional thiol for exchange. However, the peptides are sufficiently large enough to interact with neighboring disulfides and accordingly, reduce the reactivity. The extent of this screening will like depend both on the length of the sequence and its hydrophobicity, two issues that can be evaluated experimentally.

In conclusion, dendrimers based on melamine are suitable for the preparation of tetravalent displays of small molecules or peptides using thiol−disulfide exchange. The products of these reactions are readily isolated by precipitation to yield products with purities suitable for immediate use without further purification. Octavalent displays of small molecules provide materials in high yields and reasonable purities after precipitation. However, octavalent displays of peptides are more problematic, yielding in this case the hexavalent conjugate. Future studies will address these limitations of thiol−disulfide exchange; the biological activity of these compounds, toxicity and antigenicity; and the preparation of libraries of peptides.

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Supporting Information Available: Synthesis, characterization, and 2-D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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