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Synthesis of Monofunctional Curcumin Derivatives, Clicked Curcumin Dimer, and a PAMAM Dendrimer Curcumin Conjugate for Therapeutic Applications

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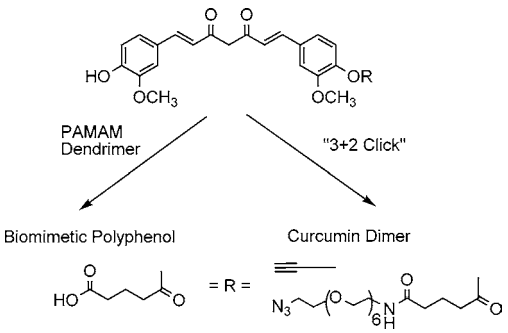
ABSTRACT



Curcumin, the primary active ingredient in the spice turmeric, was converted to reactive monofunctional derivatives (carboxylic acid/azide/alkyne). The derivatives were employed to produce a 3 + 2 azide–alkyne "clicked" curcumin dimer and a poly(amidoamine) (PAMAM) dendrimer–curcumin conjugate. The monofunctional curcumin derivatives retain biological activity and are efficient for labeling and dissolving amyloid fibrils. The curcumin dimer selectively destroys human neurotumor cells. The synthetic methodology developed affords a general strategy for attaching curcumin to various macromolecular scaffolds.

Curcuma longa commonly referred to as "turmeric" is used as a spice in South Asian cooking, as a cosmetic, and in the ancient Ayurvedic system of medicine.[1a] There has recently been tremendous interest in curcumin [(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione], the primary active ingredient in turmeric, because it has been shown to have antioxidant,[1b] anticancer,[1c] anti-inflammatory,[1d] and potent anti-Alzheimer's disease activity.[1e] Amyloid-β peptide (Aβ) aggregation is suspected to play an important role in Alzheimer's disease (AD);[2] amyloid deposits are also implicated in amyloid heart disease.[3] One of the major limitations of using curcumin as a drug is its poor water and plasma solubility; even doses as high as 8 g of curcumin per day administered to human subjects results in an average peak serum concentration of ~1.77 μM of curcumin.[4] The

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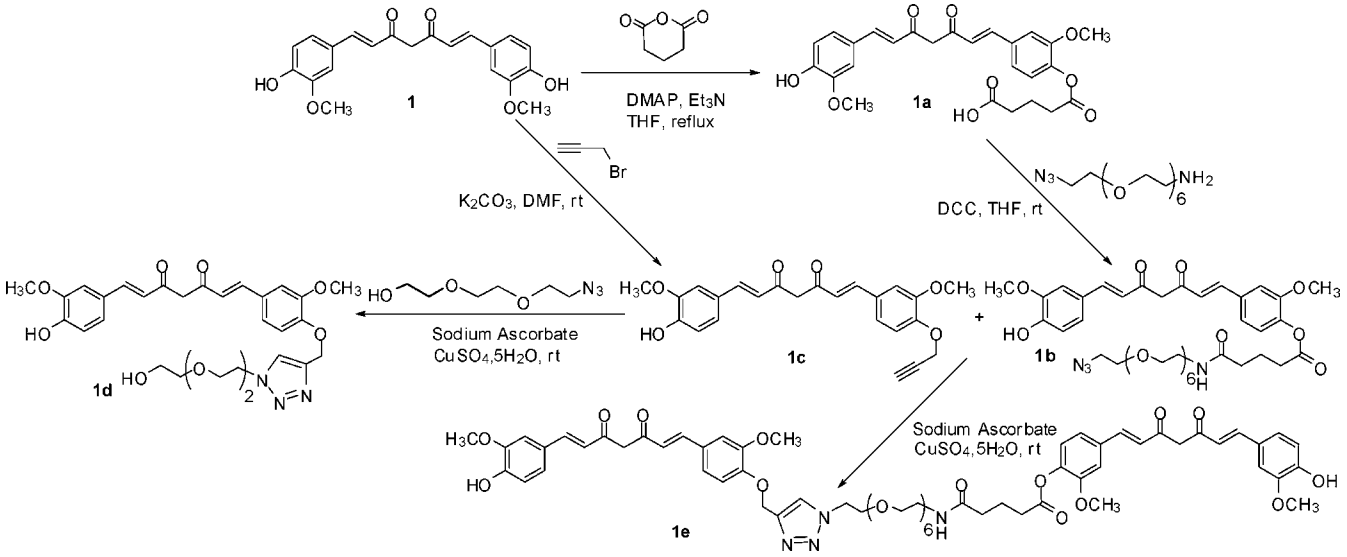
Scheme 1. Synthesis of Monofunctional Curcumin Derivatives



glucuronidation of curcumin in vivo is another possible reason for the observation of very low plasma levels of the compound.[5] The development of a synthetic methodology to produce curcumin conjugates with water-soluble polymers and targeting proteins can potentially enhance the therapeutic efficacy of curcumin. Higher molecular weight plant polyphenols have been shown to possess amplified physiological properties such as antioxidant or anticancer properties compared to their low molecular weight analogues.[6] The chemical synthesis of therapeutically relevant, well-defined high molecular weight polyphenols is very rare.

In this communication, we present a convenient route to water-soluble polyvalent curcumin conjugates via the synthesis of novel monofunctional curcumin derivatives in which one of the phenolic groups of curcumin has been chemically modified with reactive groups (azide, alkyne, and carboxylic acid) (Scheme 1). The synthesis of monofunctional curcumin derivatives affords two advantages: (a) the presence of at least one free phenolic group is necessary for the biological activity of many antioxidants such as curcumin;[1b] (b) bioconjugation and polymer modifications using monofunctional derivatives produce soluble conjugates in high yields, whereas bifunctional derivatives would result in insoluble cross-linked products.[7] To the best of our knowledge, this is the first report describing a general methodology for preparing reactive monofunctional curcumin derivatives; the unique carboxylic acid/azide/alkyne groups serve as covalent functional handles for modifying both synthetic polymers and proteins. There has been a recent report describing the multistep synthesis of a monofunctional alkyl fluoride derivative of curcumin starting from a vanillin derivative,[8] in contrast to the previous report, the approach we present here involves direct one/two-step covalent modification of curcumin to produce reactive monofunctional derivatives. A curcumin dimer was synthesized by condensing curcumin azide and curcumin alkyne using the copper-catalyzed azide−alkyne "click" reaction.[9]

Dendritic polymers are arguably the most spectacular example of a synthetic polyvalent scaffold. Dendrimers have potential applications in diverse areas ranging from material science to nanomedicine.[10] A cystamine core PAMAM dendrimer−curcumin conjugate was synthesized, and this serves as a demonstration of the utility of reactive monofunctional curcumin derivatives in the synthesis of biomimetic polyphenols. Preliminary in vitro biological studies indicate that the monofunctional derivatives of curcumin efficiently label and dissolve amyloid fibrils. The curcumin dimer selectively destroys human neurotumor cells.

The monocarboxylic acid derivative of curcumin **1a** was synthesized by reacting curcumin **1** with glutaric anhydride in the presence of base in accordance with Scheme 1. Curcumin monoazide derivative **1b** was synthesized by an amide coupling reaction between curcumin monocarboxylic acid **1a** and an amino-PEG azide using 1,3-dicyclohexylcarbodiimide (DCC) at room temperature (Scheme 1). The monoalkyne derivative of curcumin **1c** was synthesized by etherifying curcumin with propargyl bromide; K_2CO_3 was used as a base in DMF at room temperature (Scheme 1). The etherification of phenols with standard alkyl bromides requires elevated temperature and extended reaction time. In contrast, etherification involving curcumin and propargyl bromide proceeded very efficiently at room temperature; previous reports with other phenols and propargyl bromide support this observation.[9b,11] The monotriazole-PEG derivative of curcumin **1d** was synthesized by condensing the monoalkyne derivative of curcumin **1c** with azidotriethylene glycol under the Sharpless click conditions (copper(II) sulfate and sodium ascorbate). A curcumin dimer was synthesized

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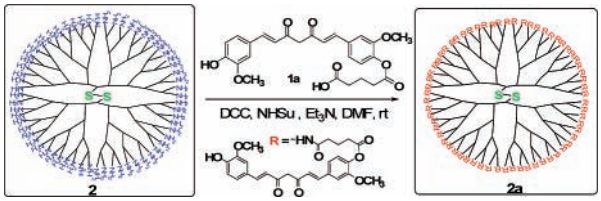
by reacting curcumin monoalkyne derivative **1c** with curcumin monoazide derivative **1b** using copper(II) sulfate and sodium ascorbate (Scheme 1). The curcumin dimer **1e** has two curcumin moieties connected via a triazole link and a PEG spacer; the dimer has two phenolic groups like the parent molecule **1**. The structures of all the curcumin derivatives **1a**, **1b**, **1c**, **1d**, and **1e** were confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectrometry (MS) (see Supporting Information). The carboxylic acid group of **1a** can be conjugated to proteins, biopolymers, and synthetic polymers; the azide **1b** and the alkyne derivative **1c** could be attached to modified proteins and polymers via the click bioconjugation reaction. It should be noted that monofunctional curcumin derivatives are necessary to produce soluble conjugates.

A Generation 4 cystamine core poly(amidoamine) dendrimer, with amine surface groups from Dendritic Nanotechnologies was coupled to curcumin monocarboxylic acid **1a** using DCC, *N*-hydroxysuccinimide (NHSu), and TEA in DMF to produce the conjugate **2a** (Scheme 2). The product

Scheme 2. Synthesis of the Dendrimer−Curcumin Conjugate



was dialyzed extensively using a 3500 MWCO membrane and further purified by a Sephadex LH20 size exclusion column. The number of **1a** units attached per dendrimer was estimated to be 37 from ^{1}H NMR by comparing the intensity of Ar−H (δ = 6.31−7.41 ppm) from the curcumin component with (−CONH−CH_2−CH_2−NHCO−) protons from the dendrimer component at δ = 3.11−3.15 ppm. The curcumin−dendrimer conjugate **2a** is freely soluble in water, and the aqueous solution has the characteristic color of curcumin (Figure 1B). The conjugate was analyzed via fast protein liquid chromatography (FPLC), using 0.1 M Na_2CO_3 (pH = 9) solution as the running buffer. The peak which eluted at the same volume as the dendrimer (275 nm) with strong absorbance at 430 nm arising from curcumin indicated that curcumin was indeed conjugated to the dendrimer (Figure 1A). Appropriate control experiments with unmodified PAMAM dendrimer and a purified noncovalent mixture of the dendrimer with **1a** were also performed (see Supporting Information). It is anticipated that the conjugate **2a** will behave differently from a small molecule in vivo and exhibit the EPR (enhanced permeability and retention) effect. The conjugate **2a** can be reduced to form two dendrons each possessing a single thiol group which could then be attached to targeting peptides/proteins to deliver plasma-soluble curcumin for several potential therapeutic applications.[1b−d]

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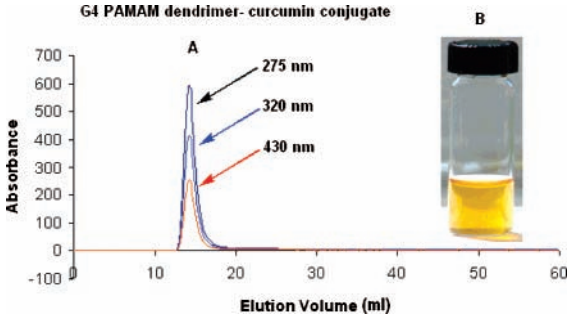

Figure 1. (A) FPLC of G4 cystamine core poly(amidoamine) dendrimer−curcumin conjugate sample **2a**; using a Hi-Prep 26/10 desalting column, 0.1 M Na_2CO_3 solution (pH = 9) was used as the running buffer. (B) Aqueous solution of the PAMAM dendrimer−curcumin conjugate **2a**.

Curcumin binds and dissolves amyloid fibrils very effectively.[1e] Preliminary studies were carried out to assess whether the monofunctional curcumin derivatives retained the ability to bind and dissolve amyloid fibrils in vitro. Human heart tissue containing intercellular amyloid was stained with Congo Red[12] according to the "Benhold's" protocol (control sample) or with curcumin monocarboxylic acid **1a** and imaged using a polarized light microscope; the images indicate that **1a** labels amyloid fibrils very effectively (Figure 2b). Curcumin and its derivatives have the advantage

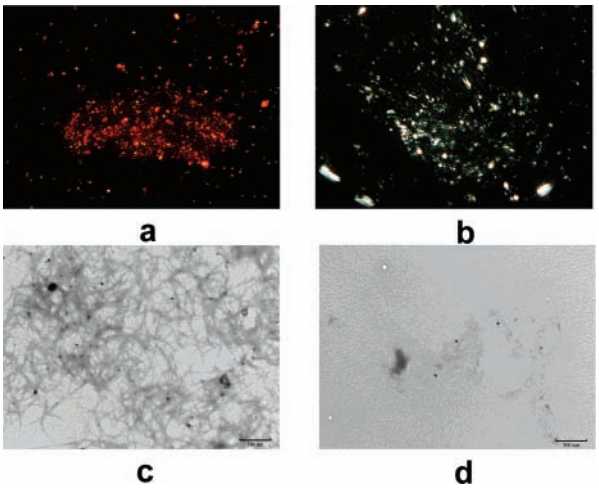

Figure 2. Human heart tissue containing intercellular amyloid stained using (a) 0.014 M Congo Red (control sample) and (b) 50 nM curcumin monocarboxylic acid **1a** imaged using a polarized light microscope under cross polarizers. (c) Transmission electron micrograph of amyloid fibrils produced by incubating Aβ 1−40 for 6 days at 37 °C. (d) No fibrils are detected by TEM when amyloid fibrils produced by incubating Aβ 1−40 for 3 days were further incubated with **1a** (8 μM) for 3 days at 37 °C. The final Aβ 1−40 concentration is the same in both c and d. The scale bar for the TEM images is 500 nm.

that they can effectively label fibrils at a much lower concentration than Congo Red: 50 nM of **1a** compared to

0.014 M for Congo Red. The ability of **1a** to dissolve amyloid aggregates (fibrils) was also evaluated. In a typical experiment, amyloid fibrils were formed by incubating Aβ 1−40 peptide. Either curcumin monocarboxylic acid **1a** or control buffer was added to the fibrils followed by visualization using transmission electron microscopy (TEM). A network of fibrils was observed in the TEM image of the control amyloid fibril sample (Figure 2c), whereas the fibrils were absent in the sample treated with **1a** (Figure 2d). This observation was further supported by UV spectroscopy (see Supporting Information). These experiments indicate that the curcumin derivatives are promising candidates for the dissolution of amyloid fibrils.

The ability of the curcumin derivatives to eliminate SHSY5Y metastatic human neurotumor cells was evaluated using a caspase-3 activation assay. The dimer **1e** was found to be the most selective derivative. As can be seen from Figure 3, both curcumin and **1e** show a concentration-dependent caspase activation; at 500 μM, curcumin is slightly better than the dimer in inducing cell apoptosis. The same assay was performed on healthy control neuronal cells at 500 μM concentration; curcumin induced considerable apoptosis, whereas the dimer **1e** induced only marginal apoptosis (Raja and Banerjee, unpublished results).

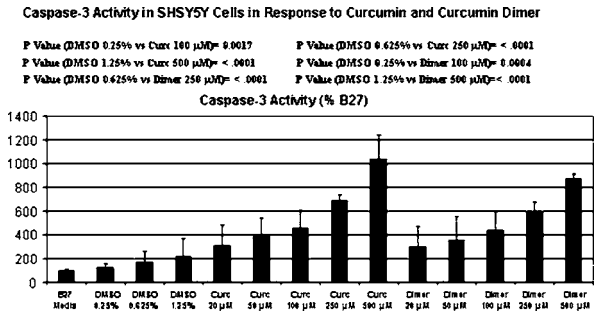

Figure 3. Caspase-3 activity in SHSY5Y metastatic human neurotumor cells in response to curcumin and curcumin dimer.

In conclusion, novel monofunctional curcumin derivatives containing reactive azide, alkyne, and carboxylic acid groups were synthesized. These derivatives were further employed to synthesize a triazole-PEG derivative, a "clicked" curcumin dimer, and a water-soluble PAMAM dendrimer−curcumin conjugate. The ability of the curcumin derivatives to stain and dissolve amyloid fibrils in vitro was evaluated. The selectivity of the curcumin dimer in destroying human neurotumor cells over curcumin suggests that it is a promising drug candidate. The biological activity studies of **2a** in vitro and in vivo are currently underway. The curcumin derivatives are currently being attached to various macromolecular scaffolds including synthetic polymers and targeting proteins to produce biomimetic polyphenols with potentially amplified biological activity.

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Supporting Information Available: Experimental procedures and characterization of synthesized compounds, FPLC chromatograms, UV spectra of amyloid samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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