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Tethered Thiazolidinone Dimers as Inhibitors of the Bacterial Type III Secretion System

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Abstract

Disruption of protein-protein interactions by small molecules is achievable but presents significant hurdles for effective compound design. In earlier work we identified a series of thiazolidinone inhibitors of the bacterial type III secretion system (T3SS) and demonstrated that this scaffold had the potential to be expanded into molecules with broad-spectrum anti-Gram negative activity. We now report on one series of thiazolidinone analogs in which the heterocycle is presented as a dimer at the termini of a series of linkers. Many of these dimers inhibited the T3SS-dependent secretion of a virulence protein at concentrations lower than that of the original monomeric compound identified in our screen.

A possible therapeutic solution to the problem of bacterial resistance to existing antibiotics is to discover drugs that will block pathogenic mechanisms rather than killing the infecting microbe. These pathogenic mechanisms include secretion systems such as the type III secretion system (T3SS) that deliver a variety of pathogen proteins using multicomponent oligomeric structures. Although many of the secreted virulence proteins are species-specific, the secretion systems are more conserved across species, indicating that disruption of such secretion systems is potentially a broad-spectrum therapeutic strategy. Because the T3SS is not required for bacterial growth *per se*, this strategy might spare commensals and limit bacterial resistance. In contrast, antibiotics that inhibit microbial growth exert a strong selection pressure for resistance.[1] In recent years the T3SS machinery has become an aggregate target for drug discovery.[2–4]

Previously our group identified a tris-aryl substituted 2-imino-5-arylidenethiazolidin-4-one, compound **1,** as a broad spectrum inhibitor of Gram-negative bacterial secretion systems (Figure 1).[5] Expansion of this chemotype enabled us to define the functional groups that could or could not be manipulated to synthetically evolve potent new analogs. Modifications at the heterocycle amido nitrogen were not only tolerated but gave rise to a series of novel dipeptide-modified congeners, for example **2** and **3**, that showed enhanced potency and physiochemical properties.[5, 6] We considered the functional architecture of the T3SS and speculated that these

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compounds might be fragments occupying only one of two inter-monomer binding sites. Prompted by this hypothesis we synthesized a bis-thiazolidinone dimer, **4**.

We analyzed dimer **4** for inhibition of the T3SS in *S. typhimurium* by monitoring secretion of a predominant substrate, SipA, into culture supernatants. Supernatant proteins were TCA precipitated, separated by SDS-PAGE and Western blotted with anti-SipA antibody. Evaluation of **4** showed a substantial increase in potency over **1**, with IC_{50} values of 5μM versus 83 μM respectively, but the poor solubility of **4** precluded further biological characterization of this compound. The significant decrease in the IC_{50} prompted us to prepare a panel of dimers, with the goal of improving the solubility of this compound and exploring the optimal inter-thiazolidinone distance and juxtaposition. For this panel, tethers were constructed that varied in length, flexibility, charge, and pendent functional groups, providing divergent presentations of the terminal thiazolidinones (Figure 2). The linear analogs **5** and **6** expand and contract overall thiazolidinone-to-thiazolidinone distance and give different placements of the amide function. In contrast to the flexible amides, the *para*, *meta*, and *ortho* diamidophenyl central cores rigidly enforce three distinct shapes (**7** – **9**). Insertion of a proline (**10**) introduces two possible kinks in the tether depending on the populations of *cis* and *trans* conformations. The five analogs that are cationic at physiological pH (**11** – **15**) can be divided into the embedded and pendent classes. Monoamine **11** is highly flexible, whereas guanidine **12** will be somewhat more rigid, and piperazine **13** is likely to assume the shape determined by a di-equatorial chair conformation. The linker in compound **14** is flexible and projects the cationic function away from the axis of the dimer. Dipeptide **15** incorporates the beneficial sequence of the potent mono-thiazolidinone **2**[5, 6] into the motif of **4**.

The syntheses of the dimers followed either a general end-to-end[7] (Scheme 1) or a center-to-outside [8] (Scheme 2) strategy. In all the analogs, the substituted thiazolidinone ring was assembled by the method of Klika.[9]

The analogs presenting pendent amino acids, **14** and **15,** were prepared by essentially linear routes (Scheme 3).

We evaluated these dimeric thiazolidinones for inhibition of the T3SS in *S. typhimurium* by again analyzing secretion of the SipA protein into culture supernatants. All of the dimeric compounds, with the exception of **7**, which was too insoluble to evaluate, were comparable to or slightly more potent than the original hit compound **1** (Table 1). These data suggest that these compounds may bind as 4-substituted thiazolidinone monomers, with the additional ring and intervening tether being innocuous but not overwhelmingly beneficial. Amide **4** is more potent than the corresponding amine **11** or guanidine **12**. This may indicate a role for the carbonyl in a critical hydrogen bond and/or result from a deleterious effect of cationic charge along the tether. The greater potency of **5** compared with **6** would argue against the carbonyl's position as a critical feature. Indeed, it is the longest linear amide **5** and the most rigidly kinked *ortho* diaminobenzene amide **9**, two uncharged analogs, that distinguish themselves among the new compounds by having potency significantly greater than **1**. Overall, compound **4** remains the most potent of the dimers, and may represent an optimum of shape, flexibility, and carbonyl placement for the cognate binding site. Alternative binding sites along the inter-protein interface are also possible and would be in agreement with the lack of a single comprehensive structure-activity trend among the dimers. The specific contribution of each individual thiazolidinone ring to the activity of the dimer remains undetermined, and we have no evidence that these rings are acting in tandem. While a definitive identification of the thiazolidinone binding site(s) will be best determined by structural biology, our results are consistent with these compounds inhibiting protein-protein interactions along a large oligomeric interface.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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7. **4 2-((2Z,5Z)-5-(4-hydroxy-3,5-dimethoxybenzylidene)-4-oxo-2-(phenylimino)thiazolidin-3-yl)-N-(6-((2Z,5Z)-5-(4-hydroxy-3,5-dimethoxybenzylidene)-4-oxo-2-(phenylimino)thiazolidin-3-yl) hexyl)acetamide (4).** To a solution of **17**[6] (24 mg, 0.053 mmol) in 0.5 mL DMF at 0 °C was added **16**[6] (25 mg, 0.061 mmol), DIEA (9.2 μL, 0.053 mmol), HOAt (7 mg, 0.053 mmol), and after 5 min, EDCI (16 mg, 0.053 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight. The crude reaction mixture was suspended in $CHCl_3$ and washed with H_2O, 1 mM citric acid, and $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude solid was purified via silica gel chromatography using a gradient from 0 to 10% MeOH in $CHCl_3$ to give **4** (5.3 mg, 0.006 mmol). ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.28–1.40 (m, 4H), 1.40–1.53 (m, 2H), 1.60–1.76 (m, 2H), 3.06–3.19 (m, 2H), 3.74 (s, 12H), 3.87 (t, *J* = 7.5 Hz, 2H), 4.47 (s, 2H), 6.83 (s, 2H), 6.84 (s, 2H), 6.98 (d, *J* = 7.5 Hz, 2H), 7.03 (d, *J* = 7.5 Hz, 2H), 7.11–7.23 (m, 2H), 7.32–7.46 (m, 4H), 7.68 (s, 1H), 7.70 (s, 1H), 8.20 (t, *J* = 5.3 Hz, 1H), 9.25 (s, 1H), 9.27 (s, 1H). ^{13}C NMR (500 MHz, DMSO-d_6, δ): 30.36, 30.48, 31.26, 33.44, 43.04, 47.03, 49.43, 60.62, 112.61, 121.91, 121.99, 125.51, 125.57, 128.07, 128.12, 129.28, 133.87, 135.72, 135.93, 142.90, 142.97, 151.90, 152.27, 152.64, 154.14, 154.25, 169.76, 170.27, 170.37. MS *m/z* 852 $[M + H]^+$, 874 $[M + Na]^+$. HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{44}H_{45}N_5O_9NaS_2$, 874.2551; found 874.2551.
8. **1-(4-(4-oxo-2-(phenylimino)thiazolidin-3-yl)butyl)-3-(2-(4-oxo-2-(phenylimino)thiazolidin-3-yl) ethyl) 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl guanidine (12).** Benzyl 4-aminobutylcarbamate (89 mg, 0.40 mmol) was dissolved in CH_2Cl_2 (2 mL), 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl isothiocyanate (125 mg, 0.40 mmol) was added, and the solution was stirred for 16 h. The reaction mixture was partitioned between EtOAc (10 mL), and water (5 mL). The organic phase was washed with 5 mL of, successively, $NaHCO_3$ and NaCl, dried, and concentrated *in vacuo* to the crude thiourea. This was dissolved in DMF (3 mL) and diisopropylethylamine (35 μL, 0.20 mmol), benzyl 2-aminoethylcarbamate HCl (28 mg, 0.14 mmol), and EDCI (35 mg, 0.12 mmol) were added. After stirring 48 h the solution was concentrated *in vacuo*, dissolved in EtOAc (10 mL), and water (10 mL) was added. The organic layer was washed with water (2×10 mL) and NaCl solution (10 mL), dried, and concentrated *in vacuo*. The residue was purified via silica gel chromatography using a gradient from 0 to 80 % EtOAc in $CHCl_3$ to give **25** (65 mg, 0.09 mmol). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.43–1.48 (m, 10H), 2.03 (s, 3H), 2.50, (s, 3H), 2.57 (s, 3H), 2.91 (s, 2H), 3.14–3.27 (m, 4H), 5.05 (s, 4H), 7.30 (s, 10H). MS *m/z* 694.4 $[M + H]^+$. Biscarbamate **25** (65 mg, 0.09 mmol) was dissolved in EtOH (5 mL), Pd-C (80 mg) was added, and hydrogen gas was bubbled through the slurry for 2 h. The resulting suspension was filtered through celite, rinsed with MeOH (5 mL), and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (4 mL), and phenyl isothiocyanate (28 μL, 0.23 mmol) and diisopropylethylamine (100 μL, 0.57 mmol)

were added. After 16 h, the solution was concentrated *in vacuo* and redissolved in THF (4 mL). Diisopropylethylamine (80 μL, 0.46 mmol) and methyl bromoacetate (25 μL, 0.27 mmol) were added, and the solution was stirred for 16 h. EtOAc (5 mL) and $NaHCO_3$ solution (5 mL) were added, the organic layer was washed with NaCl (5 mL), dried and concentrated *in vacuo*. The residue was purified via silica gel chromatography using a gradient from 0 to 80 % EtOAc in $CHCl_3$ to give the thiazolidinone (50 mg, 0.07 mmol). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.44 (s, 6H), 1.50–1.65 (m, 4H), 2.07 (s, 3H), 2.52 (s, 3H), 2.59 (s, 3H), 2.92 (s, 2H), 2.95 (br, 2H), 3.55 (br, 2H), 3.71–3.82 (m, 6H), 3.98 (br, 2H), 6.92 (t, *J* = 7.0 Hz, 4H), 7.14 (t, *J* = 7.3 Hz, 2H), 7.30–7.37 (m, 4H). MS *m/z* 776.3 $[M + H]^+$. To a solution of the thiazolidinone (11 mg, .014 mmol) and piperidine (25 μL, .253 mmol) in EtOH (3 mL) syringaldehyde (10 mg, .055 mmol) was added and the solution was heated to 90 °C for 48 h. The solution was concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2 (0.5 mL) and trifluoroacetic acid (0.5 mL), and stirred for 1 h. After removing the volatiles *in vacuo*, the residue was purified via preparative HPLC using a gradient of 10 to 40% B in 5 min, then 40 to 95% B in 20 min to give **12** (4.3 mg, .005 mmol). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.68–1.86 (m, 4H), 3.65 (t, *J* = 5.5 Hz, 2H), 3.72 (s, 12H), 3.96 (t, *J* = 6.8 Hz, 2H), 4.16 (t, *J* = 5.5 Hz, 2H), 6.62 (s, 4H), 7.00 (d, *J* = 7.5 Hz, 2H), 7.06 (d, *J* = 7.5 Hz, 2H), 7.17–7.20 (m, 4H), 7.55 (s, 1H), 7.59 (s, 1H). ^{13}C NMR (500 MHz, CD_3OD, δ): 25.82, 25.84, 27.15, 42.51, 43.41, 56.75, 56.78, 108.98, 109.02, 118.98, 122.39, 122.49, 125.55, 125.66, 126.15, 126.35, 130.48, 130.53, 133.00, 133.64, 139.79, 149.18, 149.48, 149.53, 152.27, 152.35, 157.77, 168.60. HRMS (*m/z*): $[M + H]^+$ calcd for $C_{43}H_{46}N_7O_8S_2$, 852.2844; found 852.2840.

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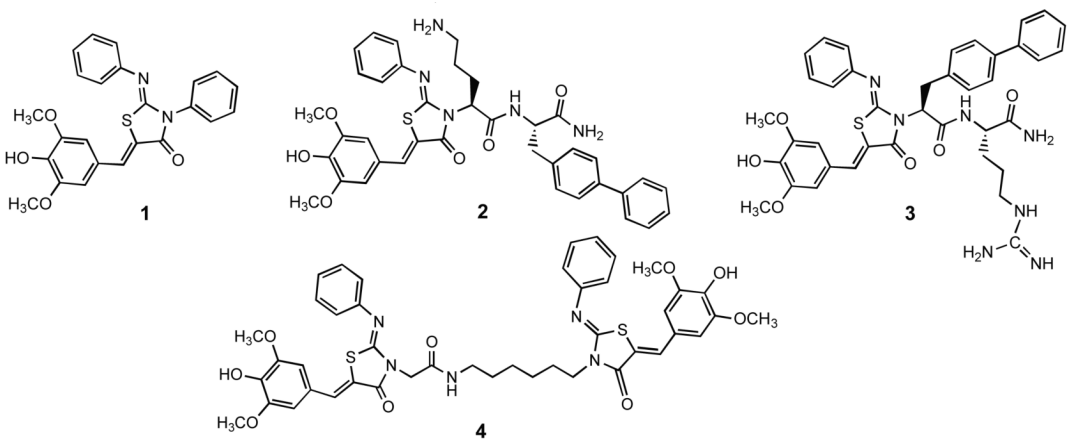


Figure 1.
The original HTS hit thiazolidinone **1**, two potent N-3 dipeptide analogs **2** and **3**, and the dimer **4**.

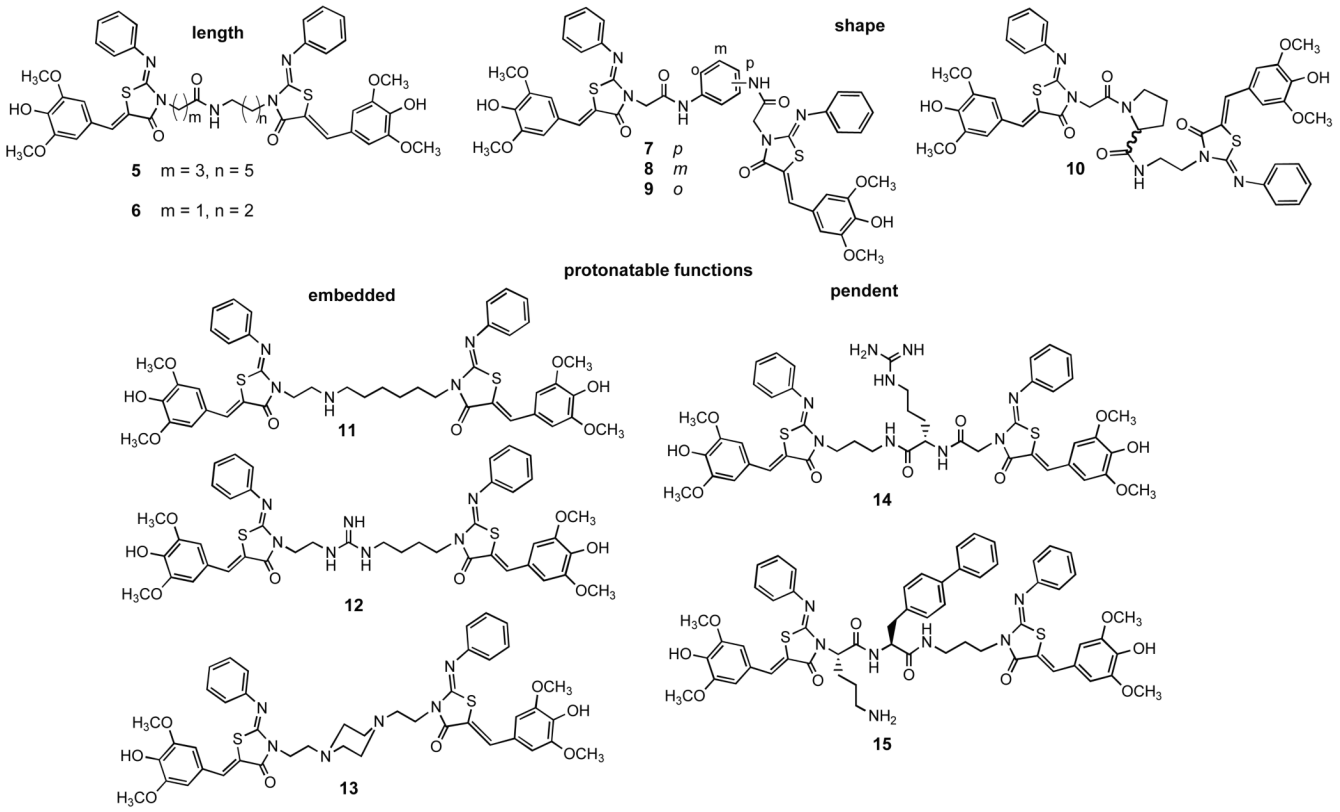
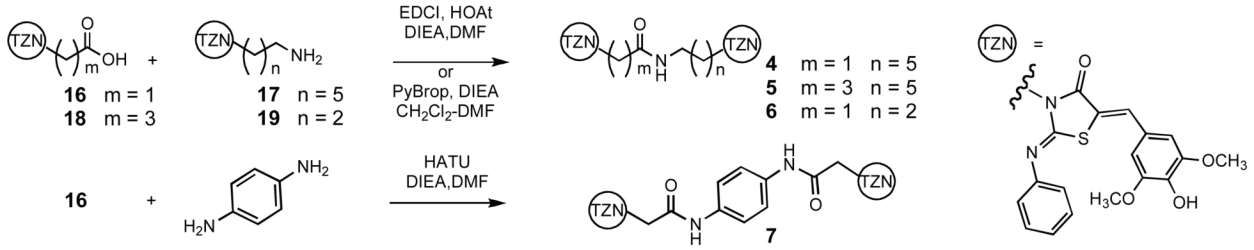
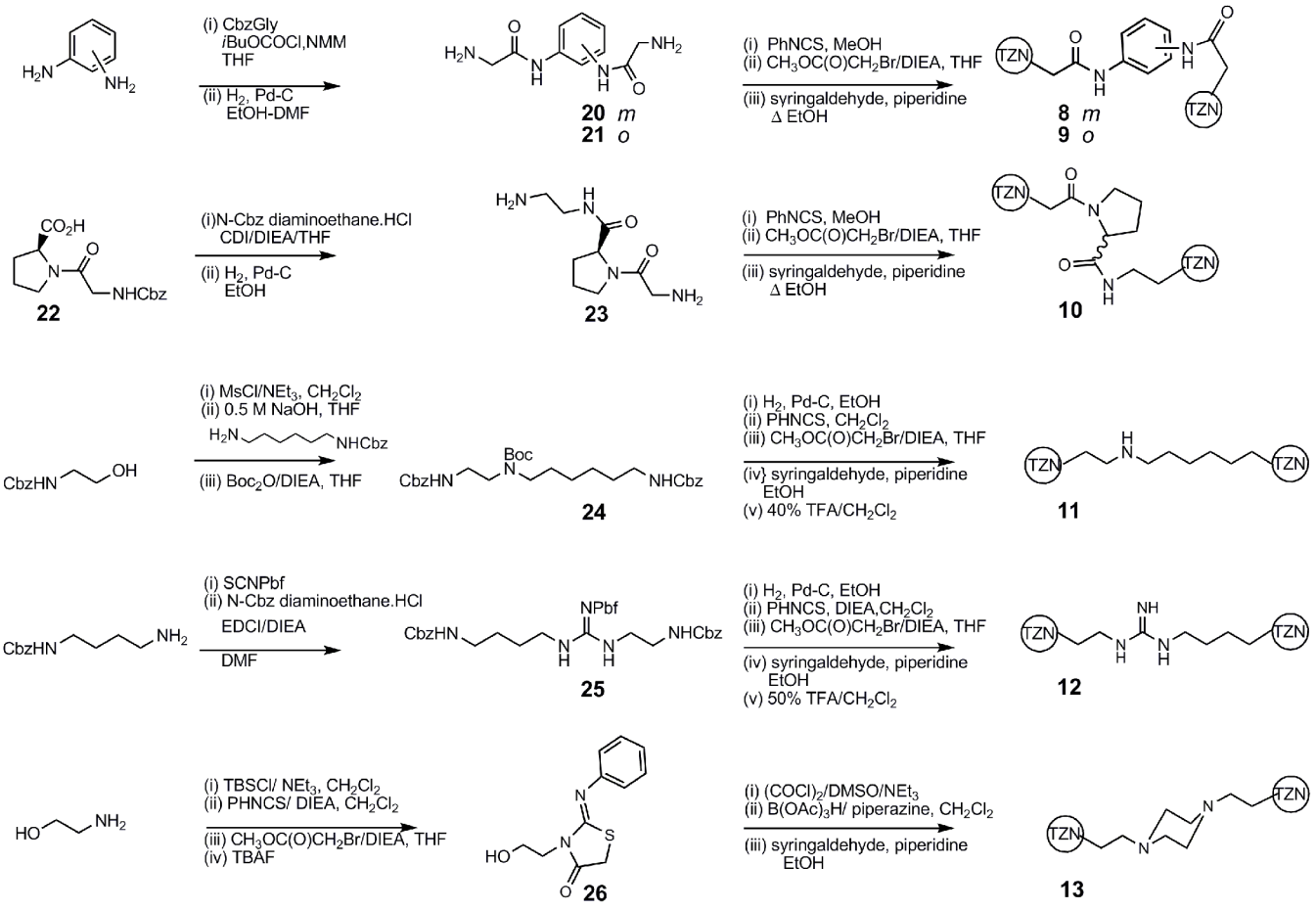


Figure 2.
Dimeric analogs **5–15** use the tether to introduce spatial and functional group properties.



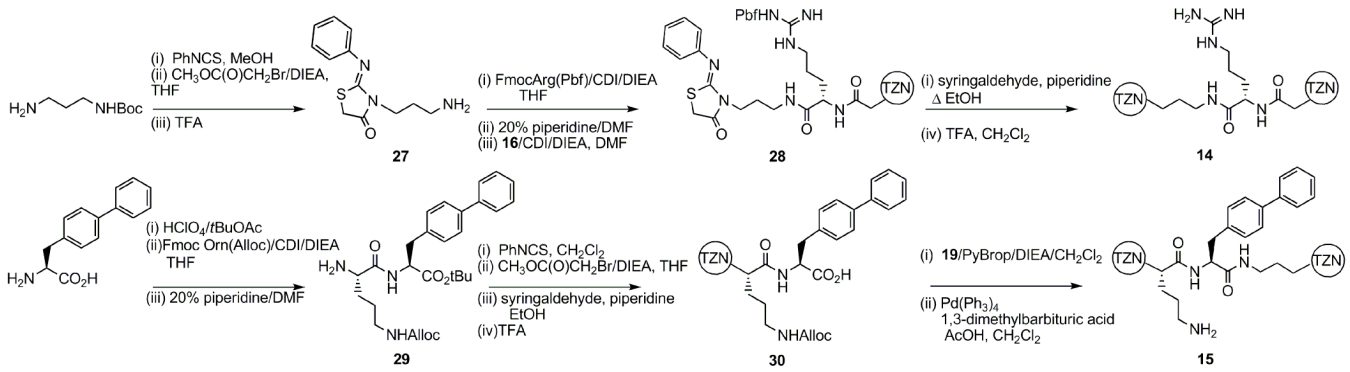
Scheme 1.

Each completely substituted thiazolidinone terminates in either an amine or a carboxylic acid that reacts with the complementary function to form the dimer.



Scheme 2.

Each tether terminates in two free amino groups that are simultaneously assembled into the substituted thiazolidinone.



Scheme 3.
The synthesis of **14** and **15.**

Table 1

Inhibition of SipA secretion by the dimeric analogs of **1.**

compound	IC_{50} (μM)
1	83
4	5
5	17
6	48
7	n.d.
8	80
9	22
10	55
11	65
12	47
13	44
14	48
15	49