Concise Synthesis of Cyclothialidine Analogues with Ring Sizes from 12 to 15: Novel Macrocyclization Protocol Involving Reductive Thiolation

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Received 7 May 2003

Abstract: The DNA gyrase inhibitor cyclothialidine was shown to be an interesting lead structure for the search of new antibacterials. During extensive work elucidating the structure-activity relations, it was demonstrated that variation of the lactone ring size of its bicyclic core was tolerated. Indeed, even ‘seco’ analogues exhibited DNA gyrase inhibitory activity. These derivatives were subsequently found to be conveniently accessible by a reductive thiolation approach. Application of this methodology to cyclic systems established an alternative, concise, and flexible synthetic access to congeners of cyclothialidine of varying ring size which so far had been prepared by Mitsunobu lactonization of the corresponding seco acids.

Key words: cyclothialidine, DNA gyrase inhibitor, antibacterials, reductive thiolation, macrocyclization

Introduction

The isolation and structure elucidation of cyclothialidine2 sparked new interest in DNA-gyrase inhibitors as antibacterial agents. From the synthesis and the biological examination of a variety of congeners of cyclothialidine, the minimal structural requirements for DNA gyrase inhibitory activity were found to be contained in a rather small partial structure.3–5 Based on this ‘minimal structure’, several new subclasses of DNA gyrase inhibitors were found comprising bicyclic compounds containing 11- to 16-membered lactone rings as well as ‘seco-compounds’ lacking the lactone moiety, best activities being displayed by the 12- to 15-membered congeners. A number of analogues were identified (Figure 1) which exhibit potent and broad antibacterial activity in vitro against Gram-positive bacteria and overcome resistance against antibacterial agents clinically used today.

The seco-compounds were readily synthesized from corresponding benzylic bromides and appropriate cysteine derivatives. However, the preparation of these benzylic bromides invoked a number of regiochemical problems. Utilization of the more readily accessible benzaldehydes as starting materials in reductive thiolations of cysteine derivatives represented a major breakthrough in the preparation of seco derivatives. The purpose of the present work is to demonstrate the usefulness of this reductive thiolation as a key step in the synthesis of cyclic 12- to 15-membered cyclothialidine analogues.

Retrosynthetic Analysis

Earlier work6,7 on the synthesis of cyclothialidine analogues utilized the Mitsunobu reaction as key step for the formation of the macrolactones (Scheme 1). The corresponding starting materials I were obtained by the alkylation of cysteine derivatives VII with properly substituted and protected benzylic bromides II. These in turn were prepared by an intrinsically unselective benzylic bromination of a dimethyl resorcinic acid derivative III. Further regiochemical problems were encountered in the synthesis of the unsymmetrically substituted resorcinic acid derivatives III from resorcinic acid IV.

Utilization of a reductive thiolation for the formation of the benzylic sulfide I allowed the use of a benzaldehydes VI rather than benzyl bromides II as starting material. This in turn solved regiochemical problems since neighboring group participation facilitated the liberation of the phenol function next to the aldehyde moiety. The development of an efficient synthesis of pseudo acid V3,5 made this approach very attractive. The Mitsunobu reaction,
however, required the protection of the phenol, prior to cyclization.

As a further improvement, we envisaged the utilization of the reductive thiolation as a key step for the macrocyclization. Alkylation of the pseudo acid V with \( N\)-\( \alpha\)-haloacylcysteine derivatives VIII should allow ready access to the key intermediates IX. Under the conditions of the reductive thiolation, cleavage of the \( S\)-trityl group should be followed by reductive cyclization directly leading to the target structures with minimal use of protective groups.

### Reductive Thiolation as a Method for the Preparation of Benzylic Sulfides

The good synthetic accessibility of pseudo acid V and its derivatives made them attractive starting materials for the preparation of benzylic sulfides I. During the formation of the dithioacetal 6 from the aldehyde 4 with \( N\)-acetylcysteine 5 in methanol in the presence of sulfuric acid with concomitant esterification (Scheme 2), the appearance of a yellow color indicated the presence of a benzylic cation. The same color change was observed when the dithioacetal 6 was dissolved in trifluoroacetic acid. Addition of triethylsilane led to the disappearance of this color. Isolation of the main reaction products revealed the formation of the desired benzylic sulfide 7 along with thiol 8. Addition of triethylsilane to an equimolar solution of aldehydes of type 1 and thiols of type 2 in trifluoroacetic acid directly led to the desired benzylic sulfides 3 in fair to good yield (Scheme 3). Reductive thiolation of simple benzaldehydes with cysteine and its derivatives under similar conditions has been reported in the literature. We explored the potential of the reductive thiolation of benzaldehydes of interest with respect to compatibility of functional groups and protective groups since we saw it as a rapid access into relevant cyclothialidine analogues. The results of this investigation are summarized in Table 1. The reaction was undisturbed by the presence of a phenolic OH group in the \( R^2\) position of the aldehyde but worked equally well when this OH group was protected as silyl ether (entries a, d, Table 1). In the thiol portion, free amino groups and free carboxylic acid groups are tolerated although the reaction tends to give better yields when amine functions are acylated (entries a, c, Table 1). Trityl groups are removed from sulfur under the condition of the reductive thiolation. (entry e, Table 1) The stereochemical composition of the benzyl sulfides was found to be determined solely by the stereochemical purity of the starting materials. The trityl protected thiol 2d (entry e, Table 1) was incorporated in the reaction both in enantiomerically pure as well as in partially racemic form. The products reflected the stereochemical composition of the thiol starting materials as evidenced by chiral GC.

### Scheme 1

![Scheme 1](image1)

### Scheme 2

![Scheme 2](image2)

### Scheme 3

![Scheme 3](image3)
Limits of the Reductive Thiolation

The hydroxy lactone V exists in a pH dependent equilibrium with its aldehyde form (Scheme 4). In the protonated state the lactone form predominates; in the salts, particularly in the N,N,N',N'-tetramethylguanidinium salt 9, only the aldehyde form can be detected by 1H NMR spectroscopy. In trifluoroacetic acid solution, V reacts with N-acetylcysteine methyl ester at room temperature both in the absence and in the presence of triethylsilane with the formation of 18 as a mixture of diastereomers. Under forcing conditions such as refluxing in trifluoroacetic acid the phthalide 19 is irreversibly formed as the only product clearly showing that lactone ring opening to form the required sulfur stabilized benzylic cation is unfavorable (Scheme 4).

Reductive Thiolation Protocol for Macrocyclization

Under the conditions of the reductive thiolation, S-detritylation occurs, however 2 equivalents of triethylsilane are required (entry e, Table 1). This finding opened the possibility to use this method as a means of macrocyclization, since for the construction of the necessary cyclization precursors IX, the thiol had to be protected in a convenient form (Scheme 5). Thus, acylation of S-tritylcysteine methyl ester (10) with the commercially available o-bromoacyl chlorides 11a–e furnished N-o-bromoacyl-S-tritylcysteine methyl esters 12a–e which reacted smoothly with the N,N,N',N'-tetramethylguanidinium salt 9 of pseudo-acid V in dimethylformamide at 50 °C to yield the cyclization precursors 13a–e. The N,N,N',N'-tetramethylguanidinium salt 9 readily precipitated from concentrated dimethylformamide solution as a stoichiometric compound. This facilitated handling and prevention of side reactions from deprotonation of the phenolic OH by using the base in situ. Depending on the chain length in the acyl portion of 12a–e the esterification reactions proceeded with moderate (n = 2 and 3) to satisfactory yields (n = 1, 4, 5). For n = 2 elimination to the dehydro derivative 16 was encountered. For n = 3 loss of the acyl group probably through an intramolecular reaction followed by hydrolysis and subsequent condensation of the liberated amine with aldehyde 13c led to considerable amounts of side product 17 (Figure 2). Treatment of a trifluoroacetic acid solution of the esters 13a–e with 2 equivalents of triethylsilane led to detritylation and reductive ring closure in satisfactory yields for the ring sizes 12–15 (14b–e), which compares favorably with the yields obtained by the more conventional Mitsunobu cyclization approach (Scheme 6, Table 2). However, for n = 1 the only isolated product from the reductive thiolation was the dimer 14a′ (Figure 3), which under the conditions chosen seems to be the thermodynamically more stable product than the 11-membered ring lactone 14a.

Table 1  Reductive Thiolation of Benzaldehydes with Cysteine Derivatives

<table>
<thead>
<tr>
<th>Entry</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>R&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Aldehyde</th>
<th>R&lt;sup&gt;4&lt;/sup&gt;</th>
<th>R&lt;sup&gt;5&lt;/sup&gt;</th>
<th>R&lt;sup&gt;6&lt;/sup&gt;</th>
<th>Thiol</th>
<th>R&lt;sup&gt;7&lt;/sup&gt;</th>
<th>Product</th>
<th>Yield (%)</th>
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<tr>
<td>a</td>
<td>Me</td>
<td>OH</td>
<td>CO₂Me</td>
<td>1a</td>
<td>CO₂Me</td>
<td>Ac</td>
<td>H</td>
<td>2a</td>
<td>OH</td>
<td>3a</td>
<td>62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>b</td>
<td>Me</td>
<td>OThDMS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CO₂Me</td>
<td>1b</td>
<td>CO₂H</td>
<td>Ac</td>
<td>H</td>
<td>2b</td>
<td>OH</td>
<td>3b</td>
<td>87&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>c</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>1c</td>
<td>CO₂H</td>
<td>Ac</td>
<td>H</td>
<td>2b</td>
<td>H</td>
<td>3c&lt;sup&gt;d&lt;/sup&gt;</td>
<td>89&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>d</td>
<td>Me</td>
<td>OH</td>
<td>CO₂Me</td>
<td>1a</td>
<td>CO₂H</td>
<td>H</td>
<td>H</td>
<td>2c</td>
<td>OH</td>
<td>3d</td>
<td>55,&lt;sup&gt;f&lt;/sup&gt; 20&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>e</td>
<td>Me</td>
<td>OH</td>
<td>CO₂Me</td>
<td>1a</td>
<td>CO₂Me</td>
<td>Ac</td>
<td>Trityl</td>
<td>2d</td>
<td>OH</td>
<td>3a</td>
<td>60&lt;sup&gt;e&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Isolated by chromatography.  
<sup>b</sup> Thexyldimethylsilyloxy.  
<sup>c</sup> Isolated by extraction and purified by crystallization.  
<sup>d</sup> See also Ref. 7  
<sup>e</sup> HPLC yield.
Conclusion

The development of a reductive thiolation protocol for the macrocyclization allows rapid access to cyclothialidine analogues with ring sizes of 12 to 15 with a minimal involvement of protective group manipulations. The new protocol compares favorable with the Mitsunobu macrolactonization approach with respect to yields, simplicity of operations and side product profiles.

Solvents and chemicals used for reactions were purchased from commercial suppliers and used without further purification. Reactions were carried out under N₂ or Ar. In the usual workup, aqueous layers were back-extracted with the organic solvent used. Organic solutions were dried with Na₂SO₄ and evaporation of solvents was performed in vacuo at 20–40 °C. Preparative chromatography: Silica gel 60 (230–400 mesh, Merck). TLC: Silica gel glass plates (Kieselgel 60 F254, Merck); detection by UV and visualization by staining with aq KMnO₄. Mp: Büchi SMP-20K apparatus, uncorrected. IR Spectra: Nicolet-20SXB spectrophotometer; data in cm⁻¹. Optical rotation: Perkin-Elmer-241 polarimeter; 10 cm, at 20 °C. ¹H NMR: Bruker-AC-250; chemical shifts δ in ppm relative to tetramethylsilane, coupling constants J in Hz. MS: EI-MS: Finnigan-MAT SSQ 700, ISP-MS: PE-Sciex API III.

Benzyl Sulfoxides 3; General Procedure

To a stirred solution of aldehyde 1a–c (2.0 mmol) and cysteine derivative 2a–d (2.0 mmol) in trifluoroacetic acid (5.00 mL) was added dropwise triethyilsilane (0.351 mL, 2.56 g, 2.2 mmol) during 5 min at 0 °C and the stirring was continued at 0 °C for 2 h. The mixture was evaporated under aspirator vacuum and the residue was purified by chromatography on silica gel with EtOAc–hexane–MeOH (40:10:1). The product fractions were collected and evaporated.

3a
Yield: 62%; white foam; [α]D 20° = –32.4 (c = 1, MeOH); GC: (Chiralpak-AD 25 cm × 4.6 mm, Nr. 19025, mobile phase 80% heptane + 20% EtOH), r.t.: 14.05 min, 99.82%.

Table 2  Comparison Mitsunobu Macrolactonization vs. Reductive Thiolation

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ring Size</th>
<th>Product (Yield, %)</th>
<th>Mitsunobu Cyclization ¹,3,5</th>
<th>Reductive Cyclization</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>11</td>
<td>14a (38)</td>
<td>14a (0)</td>
<td>14a (dimer, 62)</td>
</tr>
<tr>
<td>b</td>
<td>12</td>
<td>14b (48)</td>
<td>14b (82)</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>13</td>
<td>14c (61)</td>
<td>14c (64)</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>14</td>
<td>14d (70)</td>
<td>14d (79)</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>15</td>
<td>14e (68)</td>
<td>14e (67)</td>
<td></td>
</tr>
</tbody>
</table>

¹ Yields include cleavage of silyl protective group.
3d

The same protocol was used with S-trityl-N-acetylcysteine methyl ester 2b as starting material but the amount of triethylsilane was increased from 1.1 equiv to 2.2; yield: 20%; white powder.

3h NMR (250 MHz, DMSO-d6): δ = 1.94 (s, 3 H), 2.22–2.37 (m, 1 H), 2.60–2.75 (m, 1 H), 2.97 (dd, J = 3.0, 12.5 Hz, 1 H), 3.30–3.40 (m, 1 H), 3.76 (s, 3 H), 3.70 (m, 2 H), 3.74 (s, 3 H), 3.92 (s, 3 H), 6.53 (s, 1 H), 8.2 (br s, 1 H, OH).

MS: m/z = 208.9 (M + H)⁺.


Acylation and Esterification (10 + 11a–e → 12a–e; 12a–e + 9 → 13a–e; General procedure)

To a two-phase mixture of 0.58 g (2.15 mmol) of N-acetyl-S-tritylcysteine methyl esters 12a–e (0.2 M) in DMF was added compound 9 (1.1 equiv) and the mixture was heated under argon to 50 °C for 12 h. The reaction mixture was partitioned between H2O and EtOAc; the organic phase was washed with brine, dried (Na2SO4) and purified by chromatography on silica gel with a 1:1 mixture of n-hexane and EtOAc to afford the corresponding esters 13a–e as colorless foams (yields, see Scheme 5).

13a

IR (KBr): 3421 (br, s), 1744 (s), 1642 (s), 1516 (m), 746 (m). MS: m/z = 692.6 (M + Na)⁺.


13b

IR (KBr): 3415 (br, s), 1743 (s), 1641 (s), 1525 (m), 745 (m). MS: m/z = 640.4 (M – H)⁻.

Anal. Calcd for C35 H33 NO8 S: C, 67.38; H, 5.50; N, 1.89. Found: C, 67.25; H, 6.53; N, 1.89.

13c

IR (KBr): 3418 (br, s), 1731 (s), 1642 (s), 1516 (m), 746 (m). MS: m/z = 692.6 (M + Na)⁺.

Anal. Calcd for C35 H33 NO8 S: C, 67.38; H, 5.50; N, 1.89. Found: C, 67.25; H, 6.53; N, 1.89.
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17 (Side Product of 13c)
2 Diastereomers; yield:17%; yellow foam.
IR (KBr): 3429 (br, s), 1735 (s), 1681 (m), 1618 (s), 1489 (m), 1241 (m), 1207 (s), 745 (m), 702 cm –1 (s).

1H NMR (250 MHz, DMSO-d6): δ = 1.65–2.13 (m, 4 H), 2.05 (s, 6 H), 2.20–2.57 (m, 4 H), 2.64 (dd, J = 4.8 Hz, 4 H), 2.65 (dd, J = 13.6, 5.6 Hz, 4 H), 3.06–3.18 (m, 2 H), 3.61 (s, 3 H), 3.62 (s, 3 H), 3.68 (s, 3 H), 3.69 (s, 3 H), 3.83 (s, 6 H), 4.21–4.48 (m, 4 H), 4.50–4.65 (m, 2 H), 6.00 (d, J = 8 Hz, 2 H), 2 NH, 6.45 (s, 2 H), 7.19–7.48 (m, 30 H), 7.93 (s, 2 H), 13.68 (s, 2 H, 2 OH).

MS: m/z = 1015.9 (M + H)+.
Anal. Calcd for C60H58N2O9S2: C, 70.98; H, 5.76; N, 2.76. Found: C, 70.67; H, 5.80; N, 2.46.

Reductive Cyclization (13a–e → 14a–e); General Procedure
To a solution of the esters 13a–e (0.02 M) in trifluoroacetic acid was added dropwise triethylsilane (2.05 equiv) at 0 °C and stirred at this temperature for 2 h. The reaction mixture was partitioned between H2O and EtOAc, the organic phase was washed with brine, aq sat. NaHCO3 solution and again with brine, dried (Na2SO4) and purified by chromatography on silica gel with a 40:10:1 mixture of EtOAc–n-hexane–MeOH to afford the corresponding esters 14a–e and the dimer 14a* as white amorphous solids (yields, see Table 2).

4a (Dimer)
IR (KBr): 3407 (br, s), 1739 (s), 1666 (s), 1601 (m), 1598 (s), 1532 (s), 1250 (s), 1148 (s), 1047 cm–1 (m).

1H NMR (250 MHz, DMSO-d6): δ = 1.97 (s, 3 H), 2.82 (d, J = 6.6 Hz, 2 H), 3.65 (s, 3 H), 3.76 (d, J = 12.0 Hz, 1 H), 3.94 (d, J = 12.0 Hz, 1 H), 4.58–4.70 (m, 1 H), 4.71 (d, J = 13.2 Hz, 1 H), 4.84 (d, J = 13.2 Hz, 1 H), 6.55 (s, 1 H), 8.41 (d, J = 17.2 Hz, 1 H, NH), 9.76 (s, 1 H, OH).

MS: m/z = 737.7 (M – H)+.

14b
IR (KBr): 3380 (br, s), 1716 (s), 1666 (s), 1601 (m), 1529 (m), 1228 (m), 1150 (s), 1051 cm–1 (m).

1H NMR (250 MHz, DMSO-d6): δ = 1.90 (s, 3 H), 2.38–2.54 (m, 2 H), 2.57 (dd, J = 15.0, 3.0 Hz, 1 H), 2.84–2.99 (m, 1 H), 3.08 (dd, J = 15.0, 4.5 Hz, 1 H), 3.65 (s, 3 H), 3.60–3.80 (m, 2 H), 3.72 (s, 3 H), 4.46–4.75 (m, 3 H), 6.49 (s, 1 H), 8.57 (s, 1 H, NH), 9.68 (s, 1 H, OH).

MS: m/z = 382.5 (M – H)+.

References
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