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A one-pot preparation of N-2-mercaptobenzoyl-amino amides

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Abstract

The HIV-1 nucleocapsid (NCp7), structurally defined by zinc-binding domains, participates in crucial stages of the HIV-1 lifecycle and is mutationally nonpermissive, making it an attractive anti-HIV target. Mode of action studies have shown that the secondary structure and activity of NCp7 can be disrupted by acyl transfer from N-2-mercaptobenzoyl-amino amides. We have developed an improved one-pot reaction that affords N-2-mercaptobenzoyl-amino acids on multi-gram scales. This synthetic route allows for rapid modular construction and has greatly expanded the scope of easily accessible potential NCp7 inhibitors.

Keywords

HBUT; Amide formation; Thioester; Topical microbicide

Ettore Appella and coworkers have previously reported a class of antiviral agents that attack the highly conserved retroviral nucleocapsid protein, NCp7, as an alternative to combinational therapies for combating the hyper-mutable HIV.^{1,2,3,4} Nucleocapsid NCp7 is a short protein (72 amino acid) which is structurally defined by two zinc-binding domains and participates in crucial stages of the HIV-1 lifecycle. Since its activity is mutationally nonpermissive, disruption of NCp7 is a target for discovery of topical anti-HIV microbicides, which should not suffer from the development of resistance to treatment.^{5,6} Mode of action studies have demonstrated that acyl transfer from N-2-mercaptobenzoyl-amino amides (SAMT, general structure **1**, Figure 1) can affect one of the zinc finger motifs, disrupt the secondary structure, and result in loss of activity of NCp7.⁴ Shattock and coworkers demonstrated that suspensions of SAMT **2** provided protection in a study using rhesus macaque vaginal challenge models with mixed R5 and X4 simian-HIV infection as well as preventing transmission of HIV-1 in cell-based antiviral assays.⁷ Development of these anti-HIV compounds was hindered by the laborious original synthesis of SAMT analogues, which required 7 overall steps.^{3,8} In order to prepare larger quantities to facilitate formulation, cell-to-cell transmission assays, and additional biological studies, an improved shorter route was desired. Herein, we wish to report a one-pot preparation of N-2-mercaptobenzoyl-amino amides, which permits the facile generation of molecular diversity and provides high purity material in multi-gram quantities. The new synthetic strategy will allow us to advance SAMT molecules as anti-HIV-1 topical microbicides and further elucidate their biological activity.

Supporting Information: General experimental methods and procedures for the preparation of **5,8,11,13,15-18,20-22**, detailed characterization data, and copies of the spectral data. This material is available free of charge via the Internet at [DOI to be inserted]

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Analysis of the SAMT structure offers two convergent synthetic routes (Scheme 1), which lead to the common, commercially available, starting material of thiosalicylic acid (**3**). Both synthetic strategies would eliminate unnecessary protecting group manipulation, which would save resources and facilitate large-scale preparation. Initially, we examined each route in a stepwise fashion to determine if we could perform a 2-step preparation of the desired SAMT structures, which would be a dramatic improvement over the previously reported route. It was unclear at the outset whether it would be more advantageous to perform the amide coupling of the salicylic acid followed by thiol acylation or the reverse. We examined both routes during the course of our studies.

Initially, studies examined amide formation using thiosalicylic acid (**3**) with an unprotected thiol to prepare SAMTs without resorting to protecting group manipulation (route a, Scheme 1). Thiosalicylic acid and the hydrochloride salt of amino amides were easily coupled to form amides using HBTU and DIEA in DMF.⁹ Unfortunately, there was substantial side reaction during aqueous work up which afforded significant amounts of disulfide **8**. The formation of disulfides proved problematic during all attempts to isolate and purify the free thiol **7** with varying amounts of disulfide developing over time. Disulfides with similar functionality were originally reported by researchers at Warner-Lambert to have activity as inhibitors of HIV nucleocapsid protein zinc fingers, but were an undesired byproduct in this case.^{10,11,12,13,14} Liebeskind and coworkers have coupled alkyl- and aryl-amines to make disulfides analogous to **8** then cleaved the disulfide with sodium borohydride to access the reactive thiols (general structure **4**, R = -Me, -Et, -*t*-Bu, -aryl, -morpholino).^{15,16} Unfortunately, the free thiols of our amino-amides (general structure **7**) were exceptionally prone to disulfide formation even under anaerobic conditions and this method was unsuccessful in our system for the preparation of pure thiols and subsequent thioesters. Although we were unable to avoid the formation of disulfide, we could drive disulfide formation to completion by modification of the workup conditions.¹⁷ A facile precipitation and filtration provided pure disulfide **8**, which is sparsely soluble in most organic and aqueous solutions.

Alternatively, reversal of reaction order, with selective acylation of the thiol followed by amide coupling, would provide the desired SAMTs (route b, Scheme 1). Acylation of the free thiol was readily accomplished in high yield to afford thioesters **9** and **11**.^{18,19,20} However, the subsequent amide coupling attempts with acylated **9** were unsuccessful (Scheme 3). Curiously, the unexpected thioketene acetal **10** was isolated as a byproduct under standard conditions for amide coupling of **9**. This byproduct is the result of enolization and intramolecular trapping of acyl thioester furnishing thioketene acetal **10**, which is stable to silica gel chromatography (Scheme 3). Since benzoyl 2-mercaptobenzoic acid (**11**) lacks enolizable protons, it was envisioned that the benzoyl would not interfere with amide coupling. However, application of the standard coupling conditions to thioester **11** and β -alanine methyl ester induced benzoyl transfer to produce N-benzoyl amide **12**. The scope of this reaction was briefly probed with thioester **11** and benzylamine, which afforded amide **14** in good yield (Scheme 3). The benzoyl transfer proceeds in both the presence and absence of coupling reagent (EDC), albeit in slightly reduced yield when EDC is omitted (Scheme 3). Thioesters **9** and **11** offered interesting yet undesirable reactivity in the context of our studies, although the facile benzoyl transfer hints at the potential of these reagents in amide formation. The reactivity of a similar system has been exploited by Liebeskind and coworkers for copper catalyzed carbon-carbon bond formation between the thioester and aryl-boronic acids.^{15,16} Future studies will clarify the ability of these thioesters to act as activated esters for amide coupling.

The successful solution to our synthetic challenge was the recognition that the amide formation with free thiosalicylic acid proceeds cleanly, but that the free thiol needed to be

capped prior to deleterious disulfide formation. This synthetic plan was realized using a two-step one-pot reaction of amide coupling followed by thiol acylation to minimize disulfide by-products and avoid thioester acyl transfer (Scheme 4). The amide coupling was carried out at room temperature using thiosalicylic acid (**3**), β -alanine amide hydrochloride salt, HBTU and *i*-Pr₂EtN in DMF. After completion of the amide formation, an activated acid was added directly to the reaction mixture to acylate the intermediate thiol **7**, which provided the desired thioester **5** in modest to excellent yield after aqueous workup and recrystallization (Scheme 4).²¹ Careful optimization of reaction work-up for thioester **5** revealed a facile isolation by precipitation and recrystallization. These conditions were utilized to produce **5** on multi-gram scale (17 grams) in high yield (70%) without the need for column purification.

The one-pot preparation allows the facile variation of the amine and the acid chloride. To demonstrate the flexibility of our route, we prepared two brief series of SAMT analogues by varying the amine or the thiol acylating agents. The first series maintained the amine coupling partner with a variety of activated esters (Scheme 5). Various aryl acid chlorides were effective in the preparation of SAMTs. Alkyne **18** was prepared by trapping of the thiol with a mixture of 4-pentynoic acid and additional amide coupling agent (*iii*, Scheme 5).²² Combination of the free aliphatic carboxylic acid and HBTU forms the activated ester *in situ* to trap the thiol intermediate albeit under longer reaction times (Scheme 5). Introduction of other commercially available carboxylic acids would allow preparation of a diversity of analogues.

The second series of analogues were prepared with variation of the amine component while maintaining the acid chloride used to cap the thiol (Scheme 6). These analogues were successfully prepared using the standard condition and demonstrated that the route tolerates steric congestion and alpha-branching of the amine component. The reactions proceeded uneventfully and provided the desired analogues after purification.

The flexibility and efficiency of the synthetic route allows the rapid preparation of new and unexplored SAMT analogues (Schemes 5 & 6).²³ Key aspects of this route, which allow facile access to molecular diversity, include modularity, simple building blocks and lack of protecting group manipulation in a one-pot reaction. Our new route and expanded library of SAMT structures will be used to probe the pharmacology and structure-activity relationships of the SAMTs as anti-HIV compounds. We will report on the biological activity of these analogues in due course. This biological data will further our understanding of the acyl transfer and aid in the design of a new generation of therapeutic molecules, which are resistant to viral mutation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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17. After dilution with EtOAc, CH₂Cl₂, and water, the reaction mixture was submitted to vigorous stirring under air for 1h. This exposure led to the complete conversion of free thiol intermediate **7** into crystalline disulfide **8** which precipitated from the mixture.
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21. Some reactions were purified by passing through a plug of silica gel prior to recrystallization to remove HOBt esters. Complete experimental details are reported in the supplementary materials.
22. One can envision a sequence of initial addition of multiple equivalents of HBTU to the amine and **3** followed by subsequent addition of carboxylic acid to react with the excess HBTU. In practice, we found that excess tetramethyluronium coupling agent will react with the primary amine to provide the corresponding tetramethylguanidine which was difficult to remove without silica gel chromatography. This deleterious reactivity necessitates the sequential addition of HBTU with the free acid after the initial amide formation is complete.
23. Representative procedure for one-pot coupling. **S-(2-((3-amino-3-oxopropyl)carbamoyl)phenyl) 3,4,5-trimethoxybenzothioate (5)**. Diisopropylethylamine (36.0 mL, 204 mmol) was added to 2-mercaptobenzoic acid (9.00 g, 58.4 mmol), HBTU (23.2 g, 61.3 mmol), and β-alanine amide hydrochloride (7.63 g, 61.3 mmol) in DMF (42.0 mL). The mixture slowly became a homogenous orange solution with stirring and maintained at rt for 12h. Addition of 3,4,5-trimethoxybenzoyl chloride (27.6 g, 120 mmol) gave a suspension. Rapid dissolution of the suspended material provided an orange solution, which was followed by precipitation of an off white solid over the

course of 2h. The mixture was diluted with CH₂Cl₂ (100 mL) and H₂O (100 mL) resulting in a biphasic mixture of two clear layers. The aqueous layer was extracted with CH₂Cl₂ (4 × 200 mL). The organic layers were combined, dried (MgSO₄), and concentrated under reduced pressure to furnish an orange oil. The residue was redissolved in EtOAc (300 mL) and the solution was washed with aqueous NaHCO₃ (0.5M, 100 mL) to remove trace DMF. The EtOAc layer was chilled to 4 °C and the product crystallized to afford a white solid, S-2-(3-amino-3-oxopropylcarbamoyl)phenyl 3,4,5-trimethoxybenzothioate (**5**) (17.0 g, 40.6 mmol, 70% yield): mp 183 °C; ¹H NMR (400 MHz, DMSO) δ 8.36 (t, *J* = 5.4 Hz, 1H), 7.67 – 7.45 (m, 4H), 7.39 – 7.28 (m, 1H), 7.21 (s, 2H), 6.81 (s, 1H), 3.87 (s, 6H), 3.77 (s, 3H), 3.40 – 3.33 (m, 2H), 2.30 (t, *J* = 7.3 Hz, 2H); ¹³C NMR (100 MHz, DMSO) δ 187.8, 172.3, 167.0, 153.0, 142.4, 141.7, 136.6, 131.3, 129.8, 129.5, 128.1, 124.7, 104.4, 60.2, 56.1, 35.8, 34.8; IR (neat) 3421, 3338, 3244, 1677, 1662, 1633, 1584, 1414 cm⁻¹; HRMS (ESI) *m/z* calcd for C₂₀H₂₃N₂O₆S [M + H] 419.1277, found 419.1267.

Figure 1.
Structures of *N*-2-mercaptobenzoyl-amino amides.

Scheme 1.
Potential synthetic routes.

Scheme 2.
Initial amide coupling results.

Scheme 3.
Amide coupling with acylated substrates.

Scheme 4.
Optimized one-pot coupling.

i. HBTU, *i*-Pr₂EtN, β-ALA-NH₂•HCl, DMF, rt; *ii*. Acid chloride (2 equiv.), 2h; *iii*. pentynoic acid, HBTU, 12h.

Scheme 5.

Variation of the ester coupling partner.

Scheme 6.
Variation of the amine coupling partner.