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Selective Synthesis and Structural Elucidation of *S*-Acyl- and *N*-Acylcysteines

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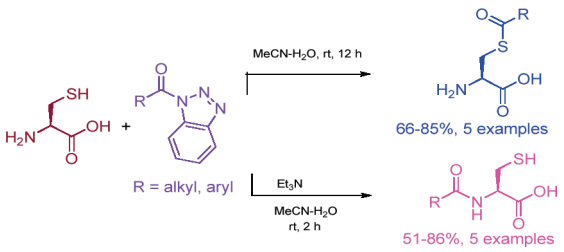

N-(Acyl)-1*H*-benzotriazoles **6a**−**f** react with L-cysteine **5** at 20 °C to give exclusively (i) *N*-acyl-L-cysteines **8a**−**e** in the presence of triethylamine in CH_3CN-H_2O (3:1), but (ii) *S*-acyl-L-cysteines **7a**−**e** in CH_3CN-H_2O (5:1) in the absence of base. Structures **7b**, **7d** and **8b**, **8d** are supported by 2D NMR spectroscopic methods including gDQCOSY, gHMQC, gHMBC, and $^1H-^{15}N$ CIGAR-gHMBC experiments. The structure of compound **8d** was also supported by single-crystal X-ray diffraction.

Cysteine-containing peptides are valuable intermediates for the synthesis of more complex peptides[1a,1b] and proteins using native chemical ligation (NCL).[1c] Native chemical ligation (NCL)[2a] is widely applied for the synthesis of proteins, uses a chemoselective reaction between two unprotected fragments, a C-terminal thioester (peptide A) **1**, and N-terminal cysteine (peptide B) **2** (Scheme 1). As shown in Scheme 1, trans-thioesterification of **1** with **2** results in intermediate **3**, after which "classical" *S* to *N* acyl transfer takes place forming an amide bond to give the new peptide **4**.[2a−e] The *S* to *N* acyl transfer from **3** to **4** is due to proximity of the amino group in **3** to the thioester functionality.

S-Acyl- and *S*-benzyloxycarbonyl cysteines are useful potential intermediates for the synthesis of cysteine and oxytocin-like peptides. Aimoto et al.[3] recently confirmed generation of an *S*-peptide via an *N* to *S* acyl shift reaction in TFA solution by a combination of ^{13}C NMR spectroscopy, reverse-phase HPLC, and MS analyses. In neat TFA, acyl chlorides selectively *S*-acylate the cysteine residues of peptides that lack serine and threonine residues without acylating amino groups.[4] Mautner et al.[5] selectively acylated thiol groups with selenol esters which react with thiols at slightly acidic pH where amino groups are protonated; lysine and histidine residues remained unreactive.

Published routes to synthesize *S*-acylcysteine derivatives have utilized (i) acyl chlorides,[4,6a,6b] (ii) acid anhydrides,[6c] (iii) selenol esters,[5] and (iv) acyl-CoAs[6d] and peptide thioesters.[2b,6e] Some of these methods have involved complex procedures and low yields.[6a,6b] We have now developed mild and efficient methods to synthesize *S*-acylcysteines.

N-Acylcysteines are found in many useful peptides[1a,1b] and proteins.[1c] They also show potential (i) as amino acid antagonists in bacteria,[7a] (ii) for quantitative determination of enantiomers of amino compounds,[7b] and (iii) as chiral ligands.[7c] Published routes to synthesize *N*-acylcysteine derivatives have also used (i) acyl chlorides[7a,8a] and (ii) acid anhydrides[8b] as well as (iii) chemical ligation[2b,2c,2e] in methods which have involved complex procedures[8a] and protection of the SH group in cysteine.[8b] Thus, mild and efficient methods to synthesize *N*-acylcysteines are desired.

N-Acylbenzotriazoles are advantageous for N-, O-, C-, and S-acylation,[9a−i] especially where the corresponding acid

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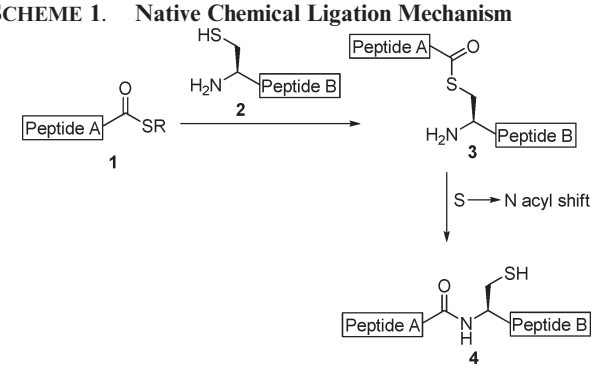
SCHEME 1. Native Chemical Ligation Mechanism



SCHEME 2. S- and N-Acylation of L-Cysteine 5

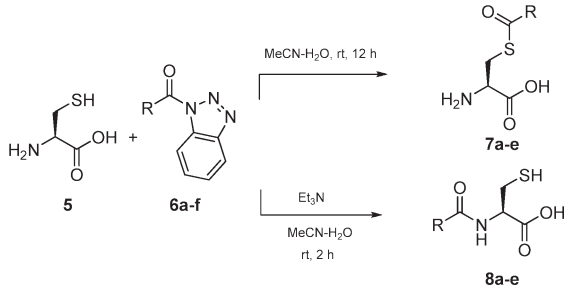


TABLE 1. Synthesis of *S*-Acylcysteines 7a−e

entry	6, R =	7, yield (%)[a]	mp (°C)
1	**6a,** phenyl	**7a**, 66	136−139
2	**6b**, 4-MeO-phenyl	**7b**, 80	166−168
3	**6c**, 4-NO_2-phenyl	**7c**, 81	158−160
4	**6d**, 2-naphthyl	**7d**, 77	167−168
5	**6e**, 4-Me-phenyl	**7e**, 85	160−161

[a]Isolated yield

chlorides are unstable or difficult to prepare.[9j,9k] Surprisingly, we located no X-ray crystal structural data for an *S*-acylcysteine and only one for an *N*-acylcysteine,[10] hence our studies also aimed to elucidate structural information for *S*-acylcysteines as well as *N*-acylcysteines. We now report the use of *N*-acylbenzotriazoles for the selective synthesis of *S*-acyl- and *N*-acylcysteines and the structural confirmation of both classes using X-ray crystallography and 2D NMR techniques.

N-Acylbenzotriazoles **6a**−**f** were prepared in 82−90% yield by the reaction of the corresponding carboxylic acid with 4 equiv of 1*H*-benzotriazole and 1 equiv of $SOCl_2$ in THF at 20 °C for 2 h.[9b] Treatment of L-cysteine **5** with *N*-acylbenzotriazoles **6a**−**e** at room temperature in MeCN−H_2O (5:1) for 12 h gave exclusively *S*-acylcysteines **7a**−**e** in yields of 66−85% as the only products isolated (Scheme 2, Table 1). Thus, at slightly acidic pH we isolated exclusively *S*-acylcysteines **7a**−**e**.

N-Acylcysteines **8a**−**e** (Scheme 2, Table 2) were synthesized by reacting L-cysteine **5** with 1 equiv of *N*-acylbenzotriazoles **6a**−**d**,**f** in the presence of 1 equiv of triethylamine in MeCN−H_2O (3:1) in yields of 51−86%, using our previously reported method.[9b] Under such basic pH conditions we isolated exclusively *N*-acylcysteines **8a**−**e**.

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TABLE 2. Synthesis of *N*-Acylcysteine 8a−e

entry	6, R =	8, yield (%)[a]	mp (°C)
1	**6a**, phenyl	**8a**, 68	oil
2	**6b**, 4-MeO-phenyl	**8b**, 67	99−101
3	**6c**, 4-NO_2-phenyl	**8c**, 86	58−60
4	**6d**, 2-naphthyl	**8d**, 85	151−152
5	**6f**, palmityl	**8e**, 51	70−72

[a]Isolated yield

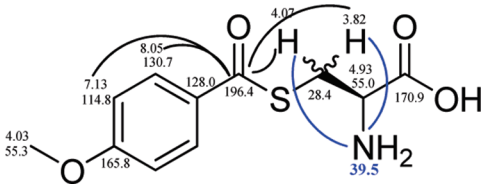

FIGURE 1. ^1H, ^{13}C, and ^{15}N chemical shift assignments of **7b**.

X-ray Crystal Structure of 8d. The structure of the *N*-naphthoyl-L-cysteine **8d** was unambiguously established by single-crystal X-ray structure determination (Figure 39 in the Supporting Information). The locations of the OH, NH, and SH hydrogen atoms were established from difference electron density maps. This is only the second example of an X-ray structure of an acylcysteine, the other being *N*-acetyl-L-cysteine.[10] In the solid state the molecules are linked by a complex system of hydrogen bonding involving interactions between adjacent amide groups and chains of hydrogen bonded carboxylic acid groups. Unfortunately, none of the *S*-acyl derivatives furnished crystals suitable for X-ray crystallography.

2D NMR Studies. The 2D NMR spectra were recorded at 500 MHz for ^1H, 125 MHz for ^{13}C, and 50 MHz for ^{15}N, equipped with a three-channel, 5-mm, indirect detection probe, with *z*-axis gradients. The chemical shifts for ^1H and ^{13}C were referenced to the residual solvent signal on the tetramethylsilane scale. The chemical shifts for ^{15}N were referenced to a frequency ratio 10.1328898482, corresponding to 0 for neat ammonia. ^1H and ^{13}C chemical shifts were assigned based on the $^1H-^1H$, one-bond and long-range $^1H-^{13}C$ couplings, seen in the gDQCOSY, gHMQC, and gHMBC spectra. $^1H-^{15}N$ CIGAR-gHMBC spectra were acquired with a pulse sequence optimized for ^{15}N as described in ref 11. The methylene protons in **7b**, **7d** and **8b**, **8d** were not stereo chemically assigned.

The spectra for *S*-(4-methoxybenzoyl)-L-cysteine **7b** were recorded in TFA-*d* at room temperature. This compound was fully assigned based on different 2D NMR experiments (Figure 1). The gHMBC (Figures 7 and 8 in the Supporting Information) experiment shows that both diastereotopic protons at 3.82 and 4.07 ppm have three-bond correlations with the *S*-CO group at 196.4 ppm. Also, the two aromatic protons at the ortho positions (8.05 ppm) and at the meta positions (7.13 ppm) show three and four-bond correlations respectively to the *S*-CO. The nitrogen chemical shift of 39.5 ppm was recorded in a CIGAR-gHMBC experiment (Figure 9 in the Supporting Information). This chemical shift was revealed through the three-bond correlation with the two diastereotopic protons at 3.82 and 4.07 ppm. The two

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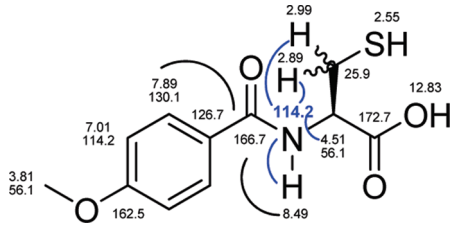

FIGURE 2. ^{1}H, ^{13}C, and ^{15}N chemical shift assignments of **8b**.

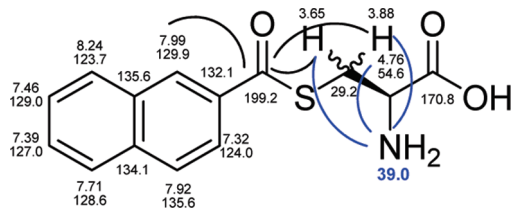

FIGURE 3. ^{1}H, ^{13}C, and ^{15}N chemical shift assignments of **7d**.

NH_2 protons and the adjacent proton at 4.93 ppm were too broad to show any correlation to the nitrogen atom. The nitrogen chemical shift appears in the expected region of free NH_2 reported in the literature.[12] These correlations further confirm that the compound exists exclusively in the *S*-acyl form.

The NMR spectra for *N*-(4-methoxybenzoyl)-L-cysteine **8b** (Figure 2) were recorded in DMSO-d_6 at room temperature. The NH proton at 8.49 ppm couples with the adjacent tertiary proton at 4.51 ppm and it appears as a doublet in the ^{1}H NMR. In gHMBC (Figure 14 in the Supporting Information), the amino proton (8.49 ppm) shows two-bond correlations with the carbonyl group at 166.7 ppm. This carbonyl group shows further correlation with the aromatic protons at 7.89 ppm, which confirms the *N*-acyl configuration. In a CIGAR-gHMBC experiment, the NH proton at 8.49 ppm shows one-bond correlation to the nitrogen atom at 114.2 ppm (Figure 15 in the Supporting Information). This chemical shift value is in agreement with the previously reported values for amide nitrogens.[12] This proton appeared as a doublet with a typical one-bond coupling constant of 90.3 Hz. This nitrogen shows three other correlations, two-bond correlation to the proton at 4.51 ppm and three-bond correlations to the protons at 2.89 and 2.99 ppm.

Compound **7d** exists exclusively in the *S*-acyl form (Figure 3) and that was easily confirmed by using different 2D NMR techniques. In a gHMBC experiment (Figures 23 and 24 in the Supporting Information), there is a three-bond correlation between the carbonyl group attached to sulfur (C at 199.2 ppm) and the aromatic proton at 7.99 ppm. Moreover, the two protons at 3.65 and 3.88 ppm have three-bond correlations with the same carbon. The assignment is confirmed by the nitrogen chemical shift (39.0 ppm) observed in a CIGAR-gHMBC experiment (Figures 25 and 26 in the Supporting Information), which falls within the range of free NH_2 reported in the literature.[12] This nitrogen has a slightly upfield value because of protonation since the spectra were taken in TFA-*d*. This chemical shift was assigned through a two-bond correlation with the proton at 4.76 ppm and the three-bond correlations with the two protons at 3.65 and 3.88 ppm.

In conclusion, we have developed a methodology for selective synthesis of *S*-acyl- and *N*-acylcysteines using *N*-acylbenzotriazoles under mild reaction conditions in good yields. At lower pH we isolated *S*-acylcysteines and at higher pH we isolated *N*-acylcysteines. Structural elucidation of *S*-acyl- and *N*-acylcysteines was achieved by using X-ray crystallography and 2D NMR techniques. ^{15}N NMR was very useful in distinguishing between the *N*- and *S*-acylcysteines because of the big difference in chemical shift between the free NH_2 and the amidic NH (75.0 ppm on average).

Experimental Section

General Procedure for Preparation of *S*-Acylcysteines 7a−e. To a solution of L-cysteine **5** (2 mmol) in H_2O (3 mL) was added a solution of the corresponding *N*-acylbenzotriazole **6** (2 mmol) in CH_3CN (15 mL). The heterogeneous mixture was then stirred at room temperature for 12 h. The solid was filtered, washed with water (3 × 10 mL), ethyl acetate (3 × 10 mL), and diethyl ether (3 × 10 mL), and dried in a desiccator under vacuum to give *S*-acylcysteines **7a−e**.

***S*-(Benzoyl)-L-cysteine (7a):** yield 66%; white microcrystals; mp 136.0−139.0 °C (lit.[6a] mp 141.0−142.0 °C); $[\alpha]^{23}_{D}$ −31.2 (*c* 1.0, MeOH−TFA (9:1)); ^{1}H NMR (300 MHz, TFA-*d*) δ 4.47 (dd, J = 15.5, 6.5 Hz, 1H), 4.73 (dd, J = 15.7, 3.4 Hz, 1H), 5.56−5.57 (m, 1H), 8.21 (t, J = 8.1 Hz, 2H), 8.41 (t, J = 7.6 Hz, 1H), 8.65−8.68 (m, 2H); ^{13}C NMR (75 MHz, TFA-*d*) δ 30.9, 57.5, 130.3, 131.9, 137.3, 138.7, 173.3, 200.8.

General Procedure for Preparation of *N*-Acylcysteines 8a−e. To a solution of L-cysteine **5** (2 mmol) and triethylamine (2 mmol) in CH_3CN-H_2O (3:1, 8 mL) was added the corresponding *N*-(acylbenzotriazole) **6** (2 mmol). The mixture was stirred at room temperature for 2 h. Solvent was removed under reduced pressure and ethyl acetate (10 mL) was added. The organic layer was washed with 2 N HCl and brine. Solvent evaporation and recrystalization (AcOEt:hexanes = 3:1) gave *N*-acylcysteines **8a−e**.

***N*-(Benzoyl)-L-cysteine (8a):**[8a] yield 68%; colorless oil; $[\alpha]^{23}_{D}$ −31.2 (*c* 3.16, MeOH); IR (neat) 3333.04, 2926.6, 1733.0, 1705.5, 1641.8, 1526.0 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51 (t, J = 9.0 Hz, 1H), 3.10−3.26 (m, 2H), 5.05−5.13 (m, 1H), 7.20 (d, J = 6.9 Hz, 1H), 7.40−7.57 (m, 3H), 7.79−7.86 (m, 2H), 8.59 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.8, 54.2, 127.5, 127.6, 127.7, 128.9, 129.0, 132.5, 168.3, 173.1. Anal. Calcd for $C_{10}H_{11}NO_3S \cdot {}^{1}/_{2}H_2O$: C, 51.27; H, 5.16; N, 5.98. Found: C, 51.64; H, 5.42; N, 6.31.

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Supporting Information Available: Materials and Methods section, characterization data for compounds **7b−e** and **8b−e**, ^{1}H NMR, gDQCOSY, gHMQC, gHMBC, and CIGAR-gHMBC spectra for compounds **7b**, **7d**, **8b**, and **8d**, ^{1}H and ^{13}C NMR spectra of compounds **7a**, **7c**, **7e**, **8a**, **8c**, and **8e** and IR spectra of compounds **7c**, **7d**, and **8a**, crystal data and structure refinement for **8d** and additional tables for **8d**, and crystallographic information file (CIFs) for **8d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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