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Preparation of N^G-Substituted L-Arginine Analogues Suitable for Solid Phase Peptide Synthesis

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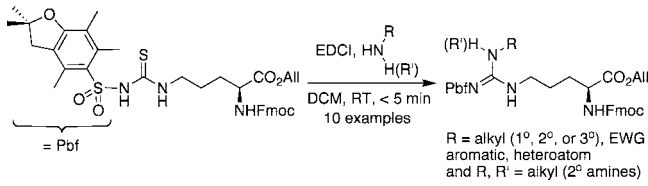

A high-yielding and concise preparation of N^G-substituted L-arginine analogues, suitably protected for use in solid phase peptide synthesis, is reported. The synthesis of each analogue employed an activated thiourea intermediate that was converted under mild conditions to the desired L-arginine analogue (10 examples, each in near quantitative yield). Subsequent allyl group removal provided each analogue in a form ideally suited for use in solid phase peptide synthesis.

L-Arginine is the most basic (pK_a 12.48) of the naturally occurring common amino acids and plays a unique role in a number of important physiological and pathophysiological processes.[1] Positively charged at neutral pH, the guanidinium group of the L-arginine side chain serves as an ion-pairing moiety in several biologically relevant molecular interactions. Proteases such as trypsin, thrombin, and Factor Xa preferentially cleave substrates containing an L-arginine residue at the P1 position.[2,3] L-Arginine is also a component of the conserved integrin receptor-binding RGD peptide motif,[4] analogues of which have been used to modulate cell adhesion.[5,6] Cell-penetrating agents have also been developed based largely upon a 9 amino acid sequence containing 6 L-arginine residues derived from the HIV-1 Tat protein.[7,8] Furthermore, homopolymers of L-arginine have been shown to mediate cellular uptake of various molecular cargoes with an efficiency and apparent mechanism of entry similar to the Tat peptide.[9,10] Aside from its numerous biological roles in peptides and proteins, L-arginine itself is also required for mammalian nitric oxide (NO) biosynthesis. The nitric oxide synthases are a class of heme proteins that oxidatively convert L-arginine to NO and L-citrulline via the intermediate N^G-hydroxy-L-arginine.[11,12] In addition, the post-translational methylation of L-arginine has recently emerged as an important regulatory mechanism in a variety of cellular processes including signal transduction, gene transcription, RNA processing, chromatin remodeling, DNA repair, viral replication, and cancer.[13−18] Enzymes implicated in both the methylation and demethylation of L-arginine have also been recently characterized.[19,20] Given the numerous roles of both L-arginine and its N^G-substituted variants in biological systems, N^G-modified L-arginine building blocks, compatible with solid phase peptide synthesis (SPPS), are desirable. We here report a high-yielding, concise, and general approach toward the preparation of a wide array of N^G-substituted L-arginine analogues suitable for use in SPPS.

While a variety of methods for the preparation of N^G-substituted guanidines have been described,[1,21−30] few can be considered of general use in providing ready access to N^G-modified L-arginine analogues suitably protected for use as building blocks in SPPS.[31] Given our recent experience in the

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(31) As an interesting alternative to the modified building block approach, Hernandez and co-workers recently described a strategy whereby guanylation of peptides was performed on-resin (method reported to be incompatible with methionine-containing peptides). Hamze, A.; Gandreuil, C.; Lisowski, V.; Andureu, F.; Fulcrand, P.; Martinez, J.; Hernandez, J. F. *Tetrahedron Lett.* **2005**, *46*, 7349–7353.

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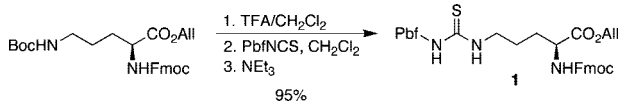
preparation of N^G-hydroxy and N^G-amino guanidines via a Cbz carbamate-activated thiourea precursor,[24,25] we sought to employ a similar approach in the current investigation (the group of Clement has also recently reported using a similar Cbz-activated thiourea approach in the racemization-free preparation of N^G-substituted L-arginine analogues[26]). Primary amines react rapidly with carbamate-protected isothiocyanates (i.e., FmocNCS[32,33] and CbzNCS[23−26]) to yield thioureas which can then be converted to carbamate-protected, N^G-substituted guanidines under mild conditions.[23] Both Fmoc and Cbz carbamates, however, are not suitable as protecting groups for the guanidine moiety of arginine in SPPS.[34] An alternative protection scheme was therefore sought that would still employ the thiourea intermediate strategy desired. In this regard the Pbf (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)- and Pmc (2,2,5,7,8-pentamethylchroman-6-sulfonyl)-protected isothiocyanates, recently developed by the groups of Fan[35] and Madalengoitia,[36] respectively, presented ideal options. While both the PbfNCS[35] and PmcNCS[36] reagents were considered suitable for the desired application, we chose to use the former given that Pbf protection is preferred for the guanidine group of arginine in SPPS.[37] To this end, the appropriately protected L-ornithine precursor was converted to Pbf-protected thiourea **1** by treatment with PbfNCS (Scheme 1).

SCHEME 1. Preparation of Pbf-Protected Thiourea 1



Of note is the particular care taken with respect to the order of reagent addition in the preparation of **1**. Previous reports involving manipulations of similarly protected L-ornithine species have described the problematic generation of 6-membered lactam side products, formed rapidly upon neutralization of the intermediate L-ornithine acid salt.[38,39] To address this concern, PbfNCS was first added to the intermediate L-ornithine TFA salt prior to the addition of NEt_3. This approach led to exclusive formation of the desired thiourea in excellent yield.

Thiourea **1** was then employed as a common precursor in the preparation of a number of N^G-substituted L-arginine analogues. Treatment of **1** with EDCI (2 equiv) followed by addition of the amine of interest (2 equiv) yielded the expected N^G-substitued L-arginine analogue (Scheme 2). In all cases investigated the conversion was complete in <5 min and in near quantitative yields (Table 1).

As shown in Table 1, a number of amines with diverse steric and electronic properties were successfully incorporated into the guanidine moiety of the corresponding protected L-arginine products (entries 1−5). Azido-substituted amines (entries 6 and 7) were incorporated to provide analogues bearing a "handle" suitable for ligation to alkynes via the popular copper(I)-mediated Huisgens 3 + 2 cycloaddition. N^G-Heteroatom-substituted L-arginine analogues (entries 8 and 9) were also readily prepared from the appropriately protected hydrazine or hydroxylamine. In addition, the successful incorporation of pyrrolidine (entry 10) indicates that (as for carbamate-activated thioureas[23,26]) the process is amenable to the use of secondary amines.

SCHEME 2. Conversion of Thiourea 1 into N^G-Substituted L-Arginine Analogue 2

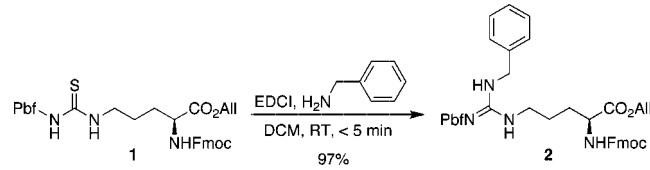
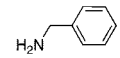
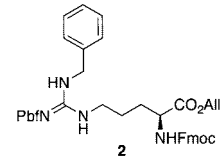
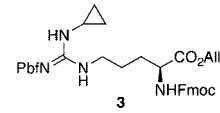
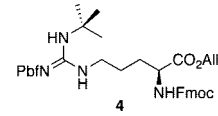
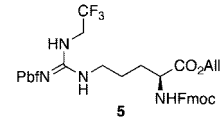
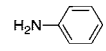
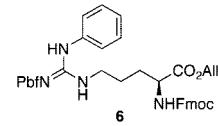
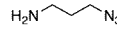
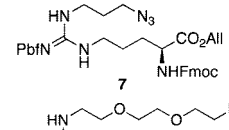
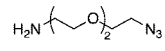
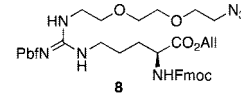
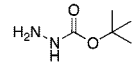
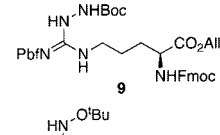
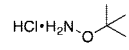
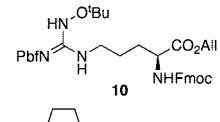
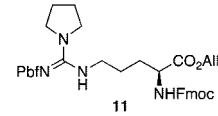


TABLE 1. N^G-Substitued L-Arginine Analogues Prepared from Thiourea 1

Entry	Amine	Product	Yield (%)
1		 **2**	97
2		 **3**	98
3		 **4**	98
4		 **5**	99
5		 **6**	98
6		 **7**	99
7		 **8**	98
8		 **9**	97
9[a]		 **10**	98
10		 **11**	99

[a] One equivalent of NEt_3 added to the reaction mixture.

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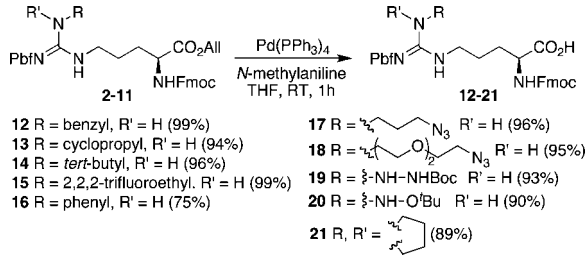
Each of the allyl-protected compounds listed in Table 1 were cleanly deprotected by using $Pd(PPh_3)_4$ and *N*-methylaniline[40] in THF to provide the corresponding N^G-modified L-arginine analogues in a form suitable for SPPS in good to excellent yields (Scheme 3).

SCHEME 3. Allyl Group Deprotection of 2−11



In summary, a concise and high-yielding methodology for the preparation of N^G-substituted L-arginine analogues suitable for use in SPPS has been developed. The generality of this methodology has been illustrated by the successful preparation of a number of new N^G-substituted L-arginine analogues, each bearing the requisite guanidine and α-amine protecting groups (Pbf and Fmoc). Access to such analogues serves to expand the repertoire of available tools for future explorations into the numerous and unique biological roles played by L-arginine and its N^G-substituted variants. In this regard, our group is currently examining a number of synthetic peptides containing N^G-modified L-arginine(s) residues for use in various biochemical studies, the results of which will be reported in due course.

Experimental Section

Details pertaining to the preparation of compounds **1**, **2**, and **12** are provided here as representative procedures.

L-Allyl 2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-5-(3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)thioureido)pentanoate (1). Fmoc-Orn(Boc)-O-All (2.23 g, 4.5 mmol) was treated with 40 mL of TFA/CH_2Cl_2 (1:1) for 1 h. After removal of TFA and CH_2Cl_2 under vacuum, the residue was redissolved in CH_2Cl_2 (20 mL) and treated with PbfNCS[35] (1.40 g, 4.5 mmol) followed by addition of NEt_3 (1.25 mL, 9.0 mmol). After stirring 1 h at ambient temperature TLC analysis revealed complete consumption of the PbfNCS with formation of the desired product (R_f 0.62, 1:1 EtOAc/hexane). The mixture was concentrated and applied directly to a silica column, eluting with 1:1 EtOAc/hexane. Following solvent removal the title compound was obtained as a white nanocrystalline foam (3.02 g, 95%). Analytical data: mp 80−82 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (br s, 1H), 8.01 (br t, J = 5.0 Hz, 1H), 7.77−7.75 (m, 2H), 7.62−7.59 (m, 2H), 7.42−7.37 (m, 2H), 7.32−7.29 (m, 2H), 5.94−5.83 (m, 1H), 5.43−5.25 (m, 3H), 4.64 (d, J = 5.5 Hz, 2H), 4.42−4.33 (m, 3H), 4.22 (t, J = 6.9 Hz, 1H), 3.56 (br d, J = 5.5 Hz, 2H), 2.95 (s, 2H), 2.56 (s, 3H), 2.48 (s, 3H), 2.10 (s, 3H), 1.98−1.52 (m, 4H), 1.46 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.8, 171.9, 161.2, 156.2, 144.1, 143.9, 141.6, 140.4, 134.5, 131.6, 128.0, 127.3, 127.1, 126.1, 125.3, 120.2, 119.5, 119.0, 87.8, 67.3, 66.5, 53.7, 47.4, 45.4, 43.1, 30.0, 28.7, 24.5, 19.5, 17.9, 12.7; HRMS (MALDI) calcd for $C_{37}H_{44}N_3O_7S_2$ [M + H]$^+$ 706.2621, found 706.2599.

L-Allyl 2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-5-(3-benzyl-2-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)guanidino)pentanoate (2). Thiourea **1** (250 mg, 0.35 mmol) was dissolved in CH_2Cl_2 (5 mL) and the solution was treated with EDCI (136 mg, 0.70 mmol) followed by benzylamine (78 μL, 0.70 mmol). Immediate TLC analysis indicated complete consumption of the starting material along with formation of a slightly more polar species (R_f 0.54, 1:1 EtOAc/hexane). The mixture was concentrated under vacuum and applied directly to a silica column eluting with a gradient of 1:1 to 2:1 EtOAc/hexane. Following solvent removal the title compound was obtained as a white nanocrystalline foam (265 mg, 97%). Analytical data: mp 63−65 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76−7.74 (m, 2H), 7.56−7.52 (m, 2H), 7.41−7.36 (m, 2H), 7.31−7.26 (m, 2H), 7.23−7.08 (m, 5H), 5.93−5.81 (m, 1H), 5.48 (br m, 1H), 5.34−5.24 (m, 2H), 4.61 (d, J = 5.5 Hz, 2H), 4.42−4.20 (m, 5H), 4.16 (t, J = 6.6 Hz, 1H), 3.38−3.01 (br m, 2H), 2.92 (s, 2H), 2.54 (s, 3H), 2.48 (s, 3H), 2.07 (s, 3H), 1.98−1.49 (m, 4H), 1.45 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 158.8, 156.5, 155.0, 144.0, 143.8, 141.5, 138.6, 133.6, 132.5, 131.6, 128.9, 128.0, 127.4, 127.3, 125.3, 124.7, 120.3, 119.5, 117.6, 86.5, 67.3, 66.5, 53.3, 47.3, 45.5, 43.5, 41.1, 30.5, 28.8, 25.4, 19.5, 18.2, 12.7; HRMS (MALDI) calcd for $C_{44}H_{51}N_4O_7S$ [M + H]$^+$ 779.3479, found 779.3473.

L-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-5-(3-benzyl-2-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ylsulfonyl)guanidino)pentanoic Acid (12). Allyl ester **2** (180 mg, 0.23 mmol) was dissolved in THF (5 mL) and treated with *N*-methylaniline (77 μL, 0.69 mmol) followed by addition of $Pd(PPh_3)_4$ (5 mol %, 0.012 mmol, 14 mg). The mixture was protected from light and stirred under N_2 (g) at room temperature for 45 min. TLC analysis at this time indicated complete consumption of the allyl ester with formation of a more polar product. The mixture was then diluted with EtOAc (50 mL) and washed with saturated NH_4Cl (25 mL). The NH_4Cl layer was further extracted with EtOAc (2 × 25 mL) and the combined EtOAc layers were dried over Na_2SO_4, filtered, and evaporated. The residue was applied to a silica column eluting first with CH_2Cl_2/MeOH (50:1) to remove the aniline impurities followed by CH_2Cl_2/MeOH/AcOH (25:1:0.1) to elute the free acid (R_f 0.21). After evaporation of product-containing fractions, the title compound was obtained as a white nanocrystalline foam (169 mg, 99%). Analytical data: mp 115−117 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.54 (br s, 1H), 7.72−7.70 (m, 2H), 7.56−7.50 (m, 2H), 7.37−7.32 (m, 2H), 7.30−7.20 (m, 2H), 7.20−7.02 (m, 5H), 5.94 (br m, 1H), 4.47−4.04 (m, 6H), 3.34−3.00 (br m, 2H), 2.87 (s, 2H), 2.48 (s, 3H), 2.41 (s, 3H), 2.03 (s, 3H), 1.98−1.47 (m, 4H), 1.43 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 158.9, 156.8, 155.1, 144.0, 143.8, 141.5, 138.6, 133.1, 132.5, 128.8, 128.0, 127.5, 127.3, 125.4, 124.8, 120.2, 117.7, 86.6, 67.4, 53.4, 47.3, 45.5, 43.4, 41.2, 29.8, 28.8, 25.2, 19.5, 18.2, 12.7; $[\alpha]_D$ −3.9 (*c* 1.2, DMF); HRMS (MALDI) calcd for $C_{41}H_{47}N_4O_7S$ [M + H]$^+$ 739.3166, found 739.3170.

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Supporting Information Available: General experimental procedures and analytical data for compounds **3**−**11** and **13**−**21** (including copies of ^{1}H and ^{13}C NMR spectra for all compounds). This material is available free of charge via the Internet at http://pubs.acs.org.

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