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Arg-Gly Thiomethylene Dipeptide Surrogates: Synthesis and Incorporation into Arg-Gly-Asp Pseudotripeptides

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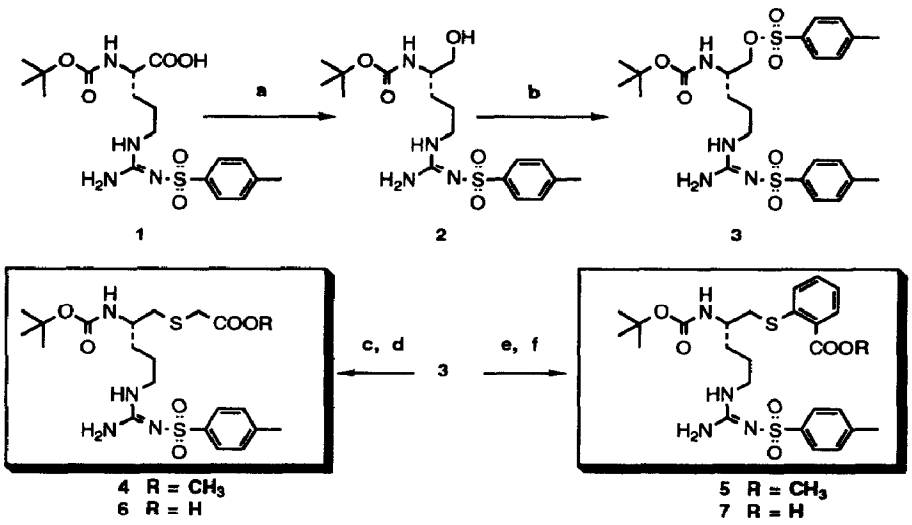
Abstract: A synthesis of Arg-Gly thiomethylene dipeptide surrogates and the incorporation of these surrogates into Arg-Gly-Asp type pseudotripeptides are described. These compounds are potentially useful for the preparation of small nonpeptide Arg-Gly-Asp peptidomimetics and may find general utility in the fields of peptide chemistry and protein engineering.

The synthesis of dipeptide surrogates and the application of these compounds for the general study of peptides[1] and also for the preparation of peptidomimetic drugs[2] has received widespread attention. Recent developments in the field of protein engineering by site directed mutagenesis[3] and by total chemical synthesis[4] insure an important and continued role for dipeptide surrogates in protein science and in drug discovery. Such studies are limited by the availability of suitably protected and structurally diverse dipeptide surrogates. As part of a program for the synthesis of cell adhesion inhibitors, we required Arg-Gly dipeptide surrogates for use in the preparation of nonpeptide mimics of the Arg-Gly-Asp binding site sequence common to the extracellular matrix proteins which mediate cell adhesion.[5] The preparation of thiomethylene Arg-Gly (ArgΨ[CH_2S]Gly) dipeptide surrogates and the incorporation of these compounds into protected Arg-Gly-Asp type pseudotripeptides are described here.

Despite popular application of amino acids as starting materials for the preparation of dipeptide surrogates and isosteres,[1] such compounds derived from arginine have been relatively few in number.[6] Protected arginine derivatives have not been commonly employed in the preparation of reduced bond dipeptide surrogates due, in part, to the formation of cyclic aminals upon attempted conversion of protected arginine to its respective amino aldehyde.[7] We reasoned that thiomethylene Arg-Gly (ArgΨ[CH_2S]Gly) dipeptide surrogates would be readily obtainable from commercially available protected arginine. Thiomethylene dipeptide surrogates have been previously described by Spatola[8] and others[9] for incorporation into biologically active peptides. To our knowledge, such compounds have not been prepared from protected arginine.

Commercially available Boc(tosyl)arginine (**1**) (Bachem) was employed for the preparation of the required thiomethylene dipeptide surrogates (Scheme 1). Diborane reduction of the protected amino acid routinely provided the corresponding alcohol **2** on a 20 g scale. Formation of the tosylate **3**[10] and subsequent displacement by the potassium salt of either methyl thioglycolate (step **c**) or methyl thiosalicylate (step **e**) gave the fully protected thiomethylene dipeptide surrogates **4** and **5** respectively. Saponification of the methyl esters afforded the free acids **6** and **7** which are suitably protected for use as dipeptide surrogates in peptide synthesis. We imagined the thiosalicylate containing surrogate **7** to be complementary to the Arg-Gly sulfide **6** in that it would be more conformationally rigid. In addition, the availability[11] of the *meta* and *para* isomers of the thiosalicylate starting material would make several regioisomeric surrogates available for structure-activity studies.

Scheme 1



Experimental Conditions:[12]

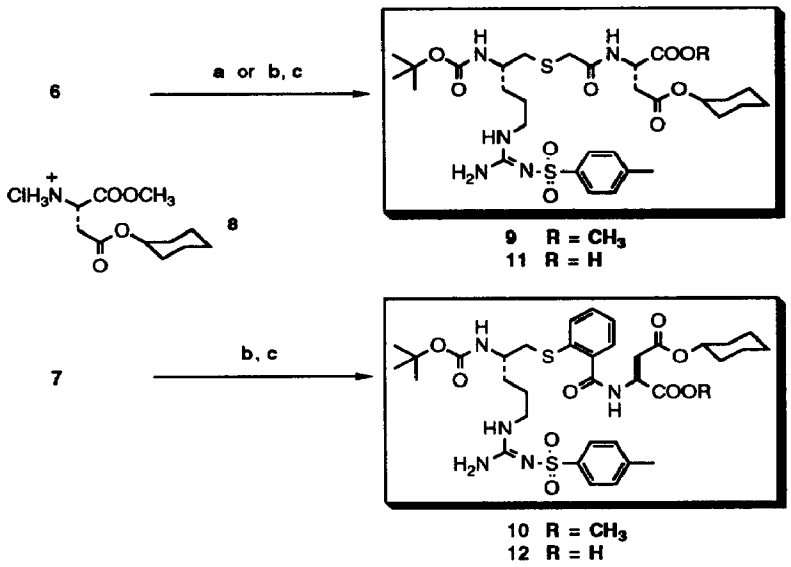
a) 3 eq BH_3 · THF, THF, -70 °C to 10 °C, 3 hr (62%). **b)** 3 eq tosyl chloride, 10 eq pyridine, $CHCl_3$, RT, 1 hr (78%). **c)** 1.5 eq $HSCH_2COOCH_3$, 1.5 eq NaH, THF, 0 °C to RT, 15 hr (72%). **d)** 1.1 eq LiOH, H_2O, MeOH, 1 hr (95%). **e)** 1.5 eq $HSC_6H_4COOCH_3$, 1.5 eq NaH, THF, 0 °C to RT, 15 hr (95%). **f)** 5 eq LiOH, H_2O, MeOH, RT, 18 hr (83%).

The thiomethylene dipeptide surrogates were employed in the preparation and characterization of fully protected thiomethylene Arg-Gly-Asp pseudotripeptides (Scheme 2). Thus, dipeptide surrogates **6** and **7** were subjected to standard solution phase peptide coupling conditions (a or b) in the presence of the methyl ester of β-cyclohexyl protected aspartic acid (**8**) to provide the fully protected pseudotripeptides **9** and **10** respectively. The β-cyclohexyl protecting group on the β-ester of the aspartic acid residue allows for selective saponification of the α-methyl esters to provide the free acid forms (**11** and **12**) of each compound. These compounds constitute Arg-Gly-Asp pseudotripeptides and are suitably protected for incorporation into peptides or proteins by the standard methods of solid phase peptide synthesis. We have used the synthesis described above to routinely prepare gram quantities of these compounds.

The sulfone form of these pseudotripeptides may be prepared as depicted in Scheme 3. The fully protected compound **4** was subjected to an excess of peracetic acid in MeOH to provide a good yield of corresponding sulfone **13**. Such sulfone analogues are complementary to the sulfide containing compounds prepared here in terms of polarity and hydrogen bonding capability and may also be used in the study of peptides and proteins.

In summary, we have prepared and characterized fully protected sulfide and sulfone containing Arg-Gly ($Arg\Psi[CH_2S]Gly$) dipeptide surrogates and Arg-Gly-Asp ($Arg\Psi[CH_2S]GlyAsp$) pseudotripeptides based on the Arg-Gly-Asp binding site sequence which is common among proteins implicated in the mediation of cell adhesion processes and inflammation. These compounds possess protecting groups commonly used in peptide synthesis (Boc, tosyl, cyclohexyl) and should find utility in the fields of peptide chemistry and protein engineering.

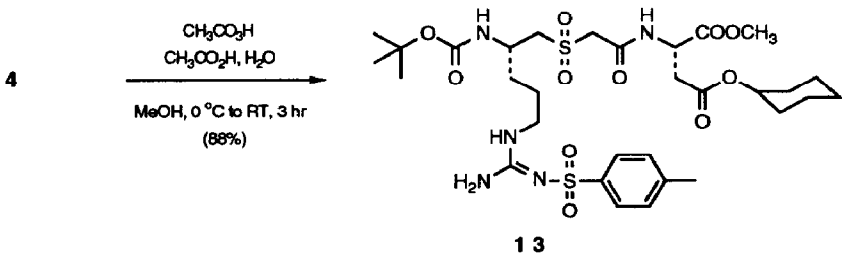
Scheme 2



Experimental Conditions:[12]

a) 1.2 eq **8**, 1.2 eq Ethyl(dimethylaminopropyl)carbodiimide · HCl (EDC), 5 eq $iPro_2NEt$, DMF (60-70%). **b)** 1.2 eq **8**, 1.2 eq Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP),[12] 5 eq $iPro_2NEt$, THF (80-90%). **c)** 1.1 eq LiOH, H_2O, THF, 2 hr, RT (85-95 %).

Scheme 3



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10) The tosylate **3** can be purified by extraction (EtOAc/1N HCl then EtOAc/sat aq $NaHCO_3$) followed by flash chromatography (30% acetone/hexane then 50% acetone/hexane) but should be used immediately as decomposition in $CDCl_3$ solution has been observed over a 48 h period.
11) Toronto Research Chemicals, Inc.
12) Fully protected compounds (**4, 5, 9, 10, 13**) are purified by extraction (EtOAc/1N HCl then EtOAc/sat aq $NaHCO_3$) followed by flash chromatography with hexane/acetone mixtures. Carboxylic acid-containing compounds (**6, 7, 11, 12**) are purified by extraction (1N HCl/$CHCl_3$) then used without further purification.

Caution: HMPA is generated in reactions that employ the BOP reagent. HMPA can be removed by additional extraction (EtOAc/sat aq LiCl). Gloves should be worn and a proper handling and disposal protocol should be followed. Reactions employing the thiol starting materials ($HSCH_2COOCH_3$ and $HSC_6H_4COOCH_3$) should be performed in an efficient fume hood due to their characteristic stench. All compounds exhibited NMR and mass spectral data in accord with their proposed structures. Spectral data for representative compounds:

Compound **4**: (colorless oil) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J = 8.0 Hz, 2H, ArH), 7.22 (d, J = 8.1 Hz, 2H, ArH), 6.46 (br s, 2H, NH_2), 6.34 (br s, 1H, NH), 4.93 (d, J = 8.7 Hz, 1H, BocNH), 3.71 (s, 3H, $COOCH_3$), 3.70 (m, 1H, CH), 3.24 (m, 3H, CH and CH_2), 2.67 (m, 2H, CH_2), 2.39 (s, 3H, tosylCH_3), 1.53-1.45 (m, 4H, 2 x CH_2), 1.41 (s, 9H, t-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 156.5, 156.1, 142.2, 140.4, 129.2, 125.9, 79.7, 52.5, 49.1, 41.1, 38.3, 33.8, 31.6, 28.3, 25.3, 21.4; MS (CI) *m/z* 503.2013 (503.1998 calcd for $C_{21}H_{34}N_4O_6S_2$, MH^+).

Compound **9**: (white foam) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (s, 1H, NH), 7.72 (d, J = 7.7 Hz, 2H, ArH), 7.22 (d, J = 8.0 Hz, 2H, ArH), 6.52 (br s, 2H, NH_2), 6.34 (br s, 1H, NH), 5.25 (d, J = 7.8 Hz, 1H, NHBoc), 4.84 (m, 1H, CH), 4.74 (m, 1H, CH), 3.71 (s, 3H, $COOCH_3$), 3.70 (m, 1H, CH), 3.25 (m, 4H, 2 x CH_2), 2.97 (dd, J = 16.7, 5.2 Hz, 1H, CH), 2.84 (dd, J = 16.7, 4.6 Hz, 1H, CH), 2.62 (d, J = 4.1 Hz, 2H, CH_2), 2.42 (s, 3H, tosylCH_3), 1.80-1.70 (m, 4H, 2 x CH_2), 1.68-1.50 (m, 6H, 3 x CH_2), 1.40 (s, 9H, t-Bu), 1.40-1.15 (m, 10H, alkyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7, 171.2, 170.1, 168.3, 156.7, 156.6, 141.9, 140.7, 129.1, 125.8, 79.5, 77.9, 73.7, 52.8, 48.9, 40.3, 38.4, 36.4, 31.4, 28.2, 25.4, 25.1, 23.5, 21.3; MS (CI) *m/z* 700.3036 (700.3138 calcd for $C_{31}H_{49}N_5O_9S_2$, MH^+).

Compound **13**: (white foam) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, J = 7.9 Hz, 1H, NH), 7.72 (d, J = 8.0 Hz, 2H, ArH), 7.22 (d, J = 8.3, 2H, ArH), 6.46 (br s, 2H, NH_2), 6.23 (br s, 1H, NH), 5.56 (s, 1H, NHBoc), 4.93-4.81 (m, 1H, CH), 4.76-4.68 (m, 1H, CH), 4.26-4.02 (m, 4H, 2 x CH_2), 3.71 (s, 3H, $COOCH_3$), 3.70 (m, 1H, CH) 3.59-3.49 (m, 1H, CH), 3.39-3.12 (m, 4H, 2 x CH_2), 2.95 (dd, J = 17.0, 5.2 Hz, 1H, CH), 2.84 (dd, J = 17.0, 4.9 Hz, 1H, CH), 2.38 (s, 3H, tosylCH_3), 1.80-1.40 (m, 4H, 2 x CH_2), 1.39 (s, 9H, t-Bu), 1.39-1.15 (m, 10H, alkyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 169.8, 161.7, 156.7, 155.7, 141.9, 140.5, 129.1, 125.8, 79.8, 77.2, 73.7, 59.2, 57.2, 52.7, 49.1, 36.0, 31.7, 31.3, 28.2, 25.1, 23.5, 21.3; MS (CI) *m/z* 732.2944 (732.3036 calcd for $C_{31}H_{49}N_5O_{11}S_2$, MH^+).

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