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A highly effective method for synthesis of N^{ω}-substituted arginines as building blocks for Boc/Fmoc peptide chemistry

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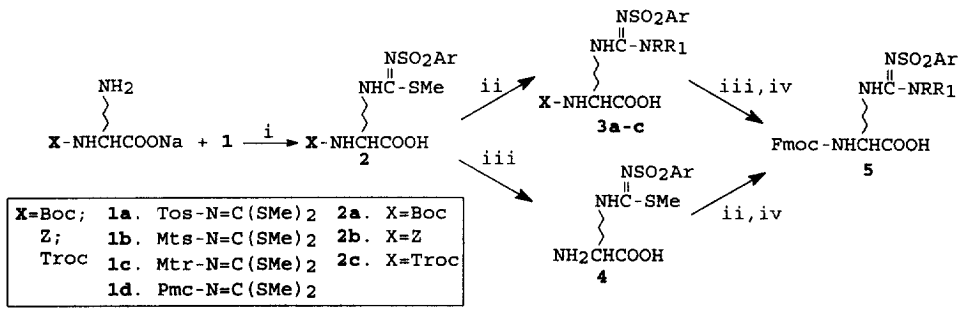
Abstract: Chemically and optically pure N^{ω}-substituted arginine derivatives were prepared with high yields in two steps, starting from N^{α}-protected ornithine and $ArSO_2N{=}C(SMe)_2$. The compounds were applied to solid phase peptide synthesis using Boc as well as Fmoc chemistries.

Keywords: N^{ω}- substituted arginine, analogues, guanylation, SPPS

Arginine-like structural motifs play important roles in biological and medical chemistry.[1,2] There are variety of methods for converting amines into guanidines (carboxamidines) either in solution or in solid phase.[3] The reactions are time-consuming, the reagents have limited flexibility and often allow only the production of unsubstituted guanidines. Furthermore, the yields are often insufficient, especially when several adjacent amino groups are functionalized.

Efficient guanylation of amines is possible *via* preformed or *in situ* generated carbodiimides.[4,5] Carbodiimides can be formed *in situ* from N-protected thioureas or S-alkylisothioureas in the presence of heavy metal ions[5] or Mukaiyama's reagent.[6] Comparing this to the methods using isolated carbodiimides, the above mentioned *in situ* approaches have numerous advantages, such as higher reactivity, high yields under mild reaction conditions and remarkable stereoselectivity. They can react with sterically or electronically unactivated amines and have been successfully used for preparing chemical libraries.[5,6]



Scheme 1, *Reagents and conditions:* **i**. H_2O/THF; 60 °C; 3-5 h; **ii**. (**A**) $HNRR_1/AgNO_3/MeCN$ or (**B**) $HgCl_2$ in MeCN/DMF, 0-60 °C, 2-12 h; **iii**. TFA, 0.5 h or HCOOH, 3.5 hs (A=Boc); Zn/AcOH, 1 h(A=Troc); 10%Pd/C in 4.4%HCOOH/MeOH, 10 min (A=Z); **iv**. Fmoc-OSu/THF/H_2O/K_2CO_3.

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Here we describe an efficient method for the synthesis of N^{ω}-modified arginines, as well as their Boc and Fmoc derivatives, with extraction and/or crystallization as the only purification steps.

Table 1. Analytical data of **X-Orn[C(=NSO$_2$Ar)NRR']-OH, (3)**

Entry	Amine[11]	HPLC Retention time (min)[7]	HPLC Purity (%)	Yield (%)	Method[11]
		3a: X=Boc; ArSO$_2$=Tos			
1	NH_3	15.8	91	89	A
2	NH_2Me	16.3	98	94	A
3	NH_2Et	17.5	90	87	A
4	NH_2cPr	18.5	85	93	A
5	NH_2Bu	20.4	98	95	A
6	NH_2But	25.9	94	95	A
7	NH_2cHex	26.9	98	96	A
8	$NH_2CH_2CH_2OH$	16.6	99	99	A
9	$NH_2CH_2CH_2NH_2$	15.4	96	82[c]	A
10	MeNHOMe. HCl	17.9	53	47[c]	A
	MeNHOMe. HCl	17.9	85	77[c]	B
11	NH_2NH_2	16.9	97	87[c]	A
12	$NH_2C_6H_4NO_2(4)$	19.2	66	60	A[a]
13	$NHMe_2$	16.8	97	94	A
14	$NHEt_2$	18.4	93	96	A
15	morpholine	17.5	98	94	A
16	$NHBu_2$	28.6	92	87	A
17	$NHcHex_2$	26.9	93	90	A
		3b: X=Z; ArSO$_2$=Mts			
18	$NH_2OH.HCl$	19.2	83	64[d]	A
	$NH_2OH.HCl$	19.2	91	83[d]	B
19	$NH_2C_6H_4NO_2(4)$	19.9	62	58	A[a]
	$NH_2C_6H_4NO_2(4)$	19.9	83	92	B[b]
20	$NH_2C_6H_4OH(4)$	20.7	87	91	B[b]
21	$NH_2C_6H_5$	22.6	95	90	A[a]
22	NH_2Me	19.7	98	95	B
23	$NHMe_2$	20.0	99	95	B
	$NHMe_2$	20.0	98	92	A
24	$NH_2CH_2C_6H_5$	22.8	95	94	B
25	NH_2NHBoc	21.9	91	95	B
26	$NH[Pr(2)]_2$	23.4	94	86	B
		3b: X=Troc; ArSO$_2$=Mts			
27	$NHMe_2$	17.4	98	95	B
		3b:X=Fmoc; ArSO$_2$=Mts			
28	$NHMe_2$	23.3	72	61[e]	B

[a] - at 60 °C, 48 hrs [b] - at 40°C; [c] - preparative RPHPLC purification; [d] - limited stability in the reaction mixture; [e] - two equivalents $[NH_2Me_2]_2CO_3$.

The synthesis of N^{ω}-modified N^{α},$N^{\omega'}$–protected arginine analogues **3** and **5** are presented in Scheme 1. The S,S-dimethylarenesulfonyliminodithiocarbonimidates **1a-d** were prepared in two steps from the corresponding sulfonyl chlorides with moderate yields (42 - 67%) and purified by crystallization. The new compounds **1b, 1c** were characterized by NMR and MS

methods.[7] The first thiomethyl group of **1** was reacted with the sodium salts of N^{α}-protected Orn, to give the key compounds **2** in near to quantitative yield.[8-10] The second thiomethyl group in the S-methylisothioureas **2**, was treated with Ag^{+}- or Hg^{2+}-salts generating an arylsulfonyl-carbodiimide.[5] This putative intermediate underwent, without isolation, a nucleophilic displacement with ammonia or various primary and secondary amines, diamines, aminoalcohols, hydrazines and hydroxylamines, etc. The above described reactions resulted in the formation of **3** with good to excellent yields[11] (Table 1). The yields were found to be largely independent of steric and electronic effects with the amines investigated. In the case of aryl amines, elevated temperatures and prolonged reaction times were necessary to accomplish the reaction. Hg^{2+}-salts gave higher yields than Ag^{+}-salts. The protected arginine analogues of **3** were isolated as noncrystalline foams, however their dicyclohexylamine salts gave amorphous crystals.[11] The N^{ω}-modified arginine analogues (with the exception of those containing free side-chain amino groups) are useful for peptide synthesis either in solution or on solid phase. The removal of Z,[12] Troc[13] or Boc-groups from **3** and the introduction of the Fmoc-group[14] were performed under standard conditions resulting in **5**. The racemization[15] was below 0.4%.

N^{α}- and side chain protected S-methylisothioureas **2**, are stable at room temperature for several months. The Z, Troc and Boc-groups can be cleaved to obtain **4** (Scheme 1). The thiomethyl group does not seem to be affected either by catalytic transfer hydrogenation or by chemical reduction.[15] An alternative synthetic route for **3** or **5**, consisting in a metal assisted displacement of thiomethyl group of **4** in the presence of primary or secondary amines, and a consecutive N^{α}-protection was tested but found to be less effective (data not shown).

The Fmoc-protected arginine analogues **5** (from **13, 21, 22**) were prepared on 30, 30 and 100 mmol scales, with yields (based on the starting material, Z-Orn-OH) of 89, 87 and 91 %, respectively. These building blocks were used for the preparation of eight dimethylarginine (***R***) or methylarginine (**r**) containing peptides[16]. The N^{ω}- methylated arginine peptides were 4 to 20 times more resistant to trypsin proteolysis than their non-methylated counterparts.

We mention that the use of the less toxic Ag^{+}-salts instead of Hg^{2+}-salts may be of advantage in some applications.

In conclusion, N^{α}-protected ornithine can be converted to various N^{α},$N^{\omega'}$-protected, N^{ω}-substituted arginine derivatives in two steps, with high chemical yields and good optical purity. The compounds were used successfully in solid phase synthesis of peptides utilizing both Boc- and Fmoc-strategies. The derivatives, **3-5**, can be used as building blocks for combinatorial libraries.[17] Such experiments are in progress.

References and notes

1. Grant, S.K., Green, B.G., Stiffey-Wilusz, J., Durette, P.L., Shan, S.K., Kozarich, J.W., *Biochemistry*, **1998**, *37*, 4174-4180.
2. Babu, B.R., Griffith, O.W., *Curr. Opin. Chem. Biol.*, **1998**; *2(4)*, 491-500.
3. For the preparation of guanidines, see: (a) Yong, Y.F., Kowalski, J.A., Thoen, J.C., Lipton, M.A., *Tetrahedron Lett.*, **1999**, *40*, 53-56; (b) Miel, H., Rault, S., *Tetrahedron Lett.*, **1998**, *39*, 1565-1568; (c) Kearney, P.C., Fernandez, M., Flygare, J.A., *Tetrahedron Lett.*, **1998**, *39*, 2663-2666 (d) Dodd, D.S., Wallace, O.B., *Tetrahedron Lett.*, **1998**, *39*: 5701-5704; (e) Jossey, J.A., Tarlton, C.A., Payne, C.E., *Tetrahedron Lett.*, **1998**, *39*, 5899-5902; (f) Lin, P., Ganesan, A., *Tetrahedron Lett.*, **1998**, *39*, 9789-9792; (g) Robinson, S., Roskamp, E.J., *Tetrahedron*, **1997**, *53*, 6697-6705; (h) Kent, D.R., Cody, W.L., Doherty, A.M., *Tetrahedron Lett.*, **1996**, *37*, 8711-8714 (i) Yamamoto, Y., Kojima, S. in "The Chemistry of Amidines and Imidates" vol 2, Chapt. 10, Patai S, Rapoport Z. eds., John Wiley & Sons: New York, **1991**.

4. Ferris, A.F., Schutz, B.A., *J. Org. Chem.*, **1963**, *28*, 71-74; (b) Drewry, D.H., Gerritz, S.W., Linn, J.A., *Tetrahedron Lett.*, **1997**, *38*, 3377-3380; (c) Nestor, J.J. Jr., Tahilramani, R., Ho, T.L., McRae,G.I., Vickery, B.H., *J. Med. Chem.*, **1988**, *31*, 65-72.

5. Kim, K.S., Qian, L., *Tetrahedron Lett.* **1993**, *34*, 7677-7680; (b) Heizmann, G., Felder, E.R., *Pepide Res.*, **1994**, *7*, 328-332; (c) Levallet, C., Lerpiniere, J., Ko, S.Y., *Tetrahedron*, **1997**, *53*, 5291-5304 and references therein.

6. Yong, Y.F., Kowalski, J.A., Lipton, M.A., *J. Org. Chem.* **1997**, *62*, 1540-1542.

7. The method of Gompper, R., Hägele, W., *Chem. Ber.*, **1966**, *99*, 2885-2899 was used; **1b**. Mts-N=C(SMe)$_2$: m.p.146 °C (from EtOH); yield 61%; Rt=26.9 min; $C_{12}H_{17}NO_2S_3$ (303.47); ESMS(MH^+) 304.00; ^{1}H NMR ($CDCl_3$): 6.93 s (2H) CH; 2.69 s (6H) 2xCH_3; 2.53 s (6H) 2xSCH_3; 2.30 s (3H) CH_3; **1c**. Mtr-N=C(SMe)$_2$: m.p.173-5 °C (from EtOH); yield 45 %; Rt= 26.9 min., see reference 9, ($C_{13}H_{19}NO_3S_3$ (333.49); ESMS(H+); 334.2; ^{1}H NMR ($CDCl_3$): 6.57 s (1H) CH; 3.85 s (3H) OCH_3; 2.72 s (3H) 6-CH_3) ; 2.58 s (3H) 2-CH_3; 2.52s (6H) 2xSCH_3; 2.13s (3H) 3-CH_3. The yields of **1c** and **1d** (42%, Rt=29.2 min:), after purification by chromatography were relatively low. The yield of **3** with **1c** and **1d** were also low (data not shown). A 10 -100 g sample was separated on LiChrospher 100 5RP18 (125x4 mm I.D.) reverse-phase column (Merck) on a Binary Waters 510 Pump System equipped with SpectraSYSTEM UV 2000 detector; linear gradient from 0% - 100% B over 33.33 min, at 1ml/min: Buffer A=0.10% TFA/H_2O; Buffer B=0.10%TFA/acetonitrile. Detection was at 214/264 nm

8. For the synthesis of **2** we used a general guanylation method (Bosin, T.R.; Hanson, R.N.; Rodricks, J.V., Simpson, R.A.; Rapoport, H., **1973**, *J. Org. Chem., 38(8)*, 1591-1600) with some modifications. A suspension of 1.0 equivalent X-Orn-OH (X= Z, Boc, Troc, Fmoc), 1.1 equivalents $NaHCO_3$ and 0.95 equivalents $ArSO_2N$=C(SMe)$_2$ applied in the form of a 0.2 - 0.3 M suspension in THF/H_2O (2.5/1.0, v/v), was stirred at 60-65 °C under nitrogen bubbling. [CAUTION, MeSH liberation !]. A solution was formed after 15 min. The reaction was complete after 3 - 5 hrs. The mixture was diluted with water (200 ml per 10 mmol) and extracted with 3x40 ml of ether. The aqueous layer was acidified to pH 3 at 4 °C with 1 M H_2SO_4 and extracted with 3x70 ml of EtOAc. The combined organic layer was washed with brine until the aqueous phase was become neutral, dried with $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The rest was used without further purification. (Orn-derivative, retention time (min), *HPLC-purity (%)*: Boc-, 25.6, *98.7*; Z-, 26.3, *>99.1*; Troc-, 26.2 , *97.9*; Fmoc-, 28.3, *72*.

9. The yields became lower if an excess of $NaHCO_3$ or diisopropylethylamine was used.

10. The regiospecific reaction of **1a** with unprotected Orn (according to Gante, J., *Angew. Chem., Int. Ed. Engl.*, **1967**, *6(10)*, 862-8) proceeds with low yield of 25-30 % **4** and requires purification by chromatography.

11. **General Procedure:** The desired amine (0.5-2.5 mmol) and 0.2 g Celite® (diatomaceous earth) were added to 0.25 mmol of **2** stirred in 6 ml MeCN. This was followed by the dropwise addition at room temperature in 5 min of **(Method A)** $AgNO_3$ (0.25mmol in 1 ml of MeCN) or **(Method B)**, $Hg(OAc)_2$ (0.28 mmol), in DMF (0.5 ml) and pyridine (0.1ml). The mixture was stirred for several hours (see Table 1), filtered and washed by MeCN (2 x 3 ml). The combined organic phases were evaporated under reduced pressure (crude **9**, **11** were characterized at this stage), dissolved in EtOAc (20 ml) and washed with 5% $KHSO_4$ and brine. The organic layer was dried ($MgSO_4$), filtered and evaporated. All the products were analyzed by HPLC and showed correct MH+ by ES-MS. All products could be crystallized as dicyclohexylammonium salts from MeOH/Et_2O/hexane(1/1/18, v/v/v) or iPrOH/Et_2O.

12. ElAmin, B., Anantharamaiah, G.M., Royer, G.P., Means, G.E., *J.Org.Chem*, **1979**, *44(19)*, 3442-3444.

13. Carson, G.F., *Synthesis*, **1981**, 268-270.

14. Siegler, G.F., Fuller, W.D., Shaturvedi, N.C., *Biopolymers*, **1983**, *22(11)*, 2157-2162.

15. **4**, H-Orn[C(=NMts)SMe]-OH, $C_{16}H_{25}N_3O_4S_2$ (387.52), ESMS(MH^+) 388.3; (MNa^+) 410.2; (MK^+) 426.1; (not covalent dimer) 775. **4**, both L and D were derivatized with Marfey's reagent (Adamson, J.G., Hoang, T., Crivici, A., Lajoie, G.A., *Anal. Biochem.*, **1992**, *202*, 210-214). The diastereomers Ld and Dd were separated by RPHPLC: gradient 0-35%B over 4 min, then 30-55%B over 40 min at 1 ml/min; retention times: Ld=26.2 min, Dd=28.1 min; The racemization was 0.2 - 0.6% including that of the starting Z-Orn-OH.

16. Peptide sequences, HPLC purities, yields, respectively: H-G***R***G***R***G***R***G***R***G***R***G***R***G-OH, 84, 77; H-GrGrGrGrGrGrGrG-OH, 80, 72; H-G***R***GGFGG***R***GGF***R***GG***R***GG-OH, 82, 77; Fmoc-GF***R****(Tos)*GG***R****(Tos)*GG-OH, 98, 79; Fmoc-***R****(Tos)*GGFGG ***R****(Tos)*G-OH; 92,64; Fmoc-FGG***R****(Tos)*GGG***R****(Tos)*GG-OH, 92, 83; Fmoc-E(But)GGFGG***R****(Tos)*GGG***R****(Tos)*GG-OH, 95, 82 and H-L***R***PG-NH_2, 92, 85. The peptides were identified with amino acid compositions and molecular masses by ESMS analyses.

17. Krchnak, V., Lebl M., *Molecular Diversity*, **1996**, *1*, 193-216.