Pergamon

Tetrahedron Letters, Vol. 37, No. 30, pp. 5409-5412, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01058-1

The Solid Phase Synthesis of a Guanidinium Based 'Tweezer' Receptor

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Abstract: An appropriately functionalised guanidine has been connected to a solid support, allowing peptide chains to be attached by conventional solid phase synthesis. This provides the basis for the solid-phase synthesis of libraries of 'tweezer' receptors for peptides with a carboxylate terminus.

A recent paper by LaBrenz and Kelly[1] described a 'tweezer' receptor[2,3] which formed a complex with a complementary peptidic guest in water, using a combination of hydrophobic and electrostatic interactions (Fig. 1). Incorporating a carboxylate binding site into a similar tweezer-like structure should lead to new receptors specifically for peptides with a carboxylate terminus (Fig. 2).[4] Furthermore, by using a solid-phase approach, libraries of receptors could be generated which would allow screening for the binding of specific peptide sequences.[5]

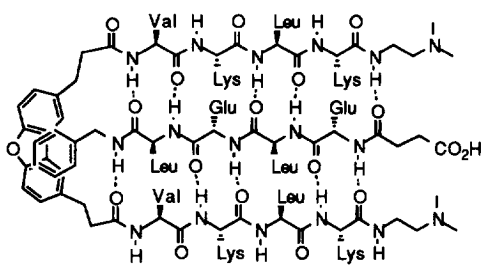


Fig. 1

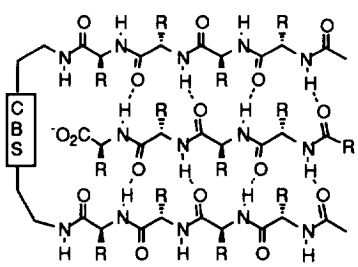
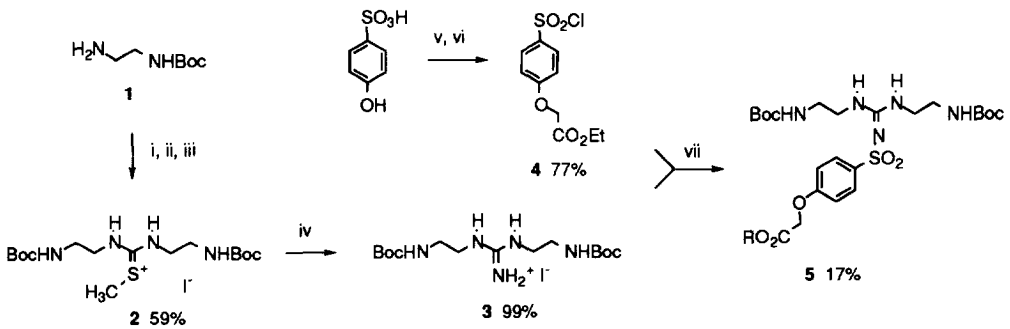


Fig. 2 CBS = carboxylate binding site

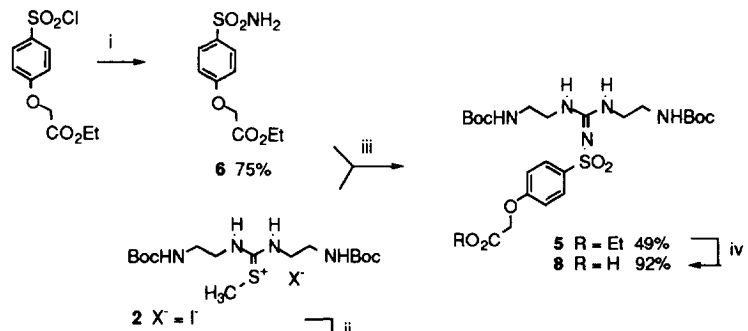
As a preliminary investigation of this approach to peptide receptors we chose a guanidinium group as the carboxylate binding site, and in this paper we describe the synthesis of an appropriately functionalised guanidine which has been connected to a solid support, allowing peptide chains to be attached by conventional solid phase synthesis.

We chose to link the guanidine unit to aminomethylpolystyrene resin[6] via an arylsulphonamide, allowing cleavage from the resin with strong acid,[7] while simultaneously providing an effective protecting group for the basic guanidine functionality. To this end, mono-*tert*-butyloxycarbonyl protected ethylene diamine **1** was converted to the thiouronium salt **2** in three steps via the isothiocyanate, in good overall yield (Scheme 1) and condensed with ammonia to give the diprotected guanidinium derivative **3**, following established literature procedures.[8] Reaction of **3** with the sulphonylchloride **4**, derived from 4-hydroxybenzene sulphonic acid, gave the desired guanidine derivative **5**, but in low yield, with products resulting from disulphonylation and sulphonylation at the more substituted guanidine nitrogens proving unavoidable.



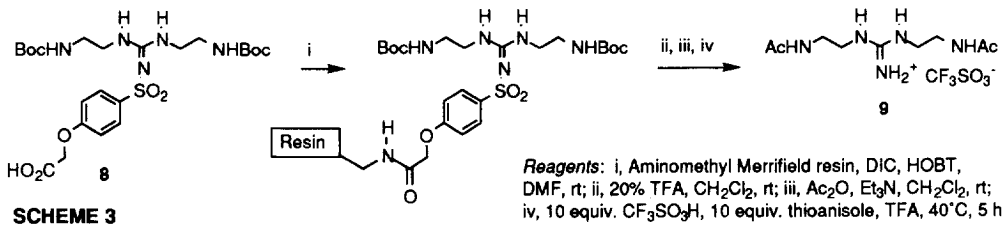
SCHEME 1 *Reagents*: i, Cl_2CS, K_2CO_3, $CHCl_3$/ H_2O, reflux; ii, 1, EtOH; iii, MeI, acetone, rt; iv, NH_3, MeOH, 70 °C, sealed tube; v, $BrCH_2CO_2Et$, K_2CO_3, DMF, rt; vi, $SOCl_2$, toluene, 100 °C; vii, NaOH, acetone, pH 10, rt

Conversion of sulphonyl chloride to the corresponding sulphonamide **6** and condensation with the thiouronium salt **7**[9,10] provided a more efficient route (Scheme 2), giving the desired guanidine derivative **5** in 49% yield. Hydrolysis of the ethyl ester gave the corresponding acid **8**.



SCHEME 2 *Reagents*: i, NH_3, EtOH, rt; ii, NH_4PF_6, CH_2Cl_2, rt; iii, DIPEA, toluene, 110°C; iv, LiOH, H_2O, dioxane, rt

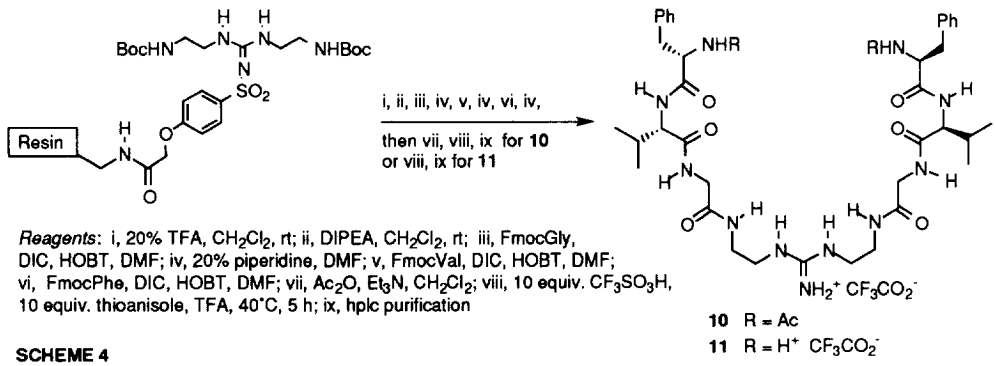
The acid **8** was coupled to aminomethylpolystyrene resin using diisopropylcarbodiimide (DIC), monitoring the reaction to completion using the quantitative ninhydrin test[11] (Scheme 3). Selective cleavage of the Boc protecting groups was carried out with 20% TFA in CH_2Cl_2, and in order to test the cleavage of the guanidine from the resin, the resulting diamine was acetylated. Cleavage of the guanidine from the solid support was successfully performed with triflic acid in TFA at 40°C, using thioanisole as a scavenger,[7] to give the diacetylated product essentially pure after trituration of the crude with diethylether.



SCHEME 3

Reagents: i, Aminomethyl Merrifield resin, DIC, HOBT, DMF, rt; ii, 20% TFA, CH_2Cl_2, rt; iii, Ac_2O, Et_3N, CH_2Cl_2, rt; iv, 10 equiv. CF_3SO_3H, 10 equiv. thioanisole, TFA, 40°C, 5 h

In order to establish the utility of the linker for the preparation of tweezer molecules we constructed a bis(tripeptide) derivative using Fmoc solid-phase chemistry[12] (Scheme 4). Removal of the final Fmoc group allowed the bis(tripeptide) to be acetylated and cleaved from the solid support, as before, to give **10**, or alternatively, cleaved as the bisammonium salt **11**. Both of the bis(tripeptides) were obtained >90% pure after trituration with diethyl ether, but were further purified by RP C-18 HPLC and characterised by MALDI-TOF-MS, electrospray MS and by NMR.[13]



Reagents: i, 20% TFA, CH_2Cl_2, rt; ii, DIPEA, CH_2Cl_2, rt; iii, FmocGly, DIC, HOBT, DMF; iv, 20% piperidine, DMF; v, FmocVal, DIC, HOBT, DMF; vi, FmocPhe, DIC, HOBT, DMF; vii, Ac_2O, Et_3N, CH_2Cl_2; viii, 10 equiv. CF_3SO_3H, 10 equiv. thioanisole, TFA, 40°C, 5 h; ix, hplc purification

10 R = Ac
11 R = H^+ $CF_3CO_2^-$

SCHEME 4

In conclusion we have successfully developed a synthetic strategy for the synthesis of a guanidinium derived tweezer receptor. This strategy should allow the synthesis of more rigid or capped, macrocyclic structures for peptide recognition, and ultimately libraries of such structures. Such work is now underway.

Acknowledgements: We thank the EPSRC for supporting this work (GR/K19938) and Ian R Marsh for assistance with the solid-phase chemistry.

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10. Attempted condensation of the sulphonamide **6** with the iodide salt **2** gave only low yields of the desired product **5**, with substantial amounts of the corresponding thiourea being formed as a result of demethylation.
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13. Data for **10**: ^{1}H NMR (300 MHz, CD_3OD) δ: 7.29 (10H, m, Ph), 4.71 (2 H, dd, J = 5, 9 Hz, C*H*CH_2Ph), 4.13 (2 H, d, J = 7 Hz, C*H*$CHMe_2$), 3.93 (2 H, d, J = 16 Hz, CH_AH_BCO), 3.81 (2 H, d, J = 16 Hz, CH_AH_BCO), 3.41 (4 H, m, CH_2CH_2), 3.35 (4 H, m, CH_2CH_2), 3.17 (2 H, dd, J = 5, 14 Hz, CH_ACH_BPh), 2.92 (2 H, dd, J = 9, 14 Hz, CH_ACH_BPh), 2.16 (2 H, m, C*H*Me_2), 1.97 (6 H, s, MeCO) 1.01 (3 H, d, J = 7 Hz, MeCH), 1.00 (3 H, d, J = 7 Hz, MeCH); ^{13}C NMR (75 MHz, CD_3OD) δ: 174.2, 174.1, 173.8, 172.1, 157.6, 138.1, 130.2, 129.4, 126.2, 60.9, 56.4, 43.5, 41.9, 39.2, 38.4, 31.3, 22.0, 19.6, 18.8; MS (electrospray) 836.5 $(M+H)^+$.
Data for **11**: ^{1}H NMR (300 MHz, CD_3OD) δ: 7.35 (10H, m, Ph), 4.28 (2 H, dd, J = 5, 8 Hz, C*H*CH_2Ph), 4.23 (2 H, d, J = 7 Hz, C*H*$CHMe_2$), 3.96 (2 H, d, J = 17 Hz, CH_AH_BCO), 3.88 (2 H, d, J = 17 Hz, CH_AH_BCO), 3.50 - 3.40 (8 H, m, CH_2CH_2), 3.32 (2 H, dd, J = 5, 14 Hz, CH_ACH_BPh), 3.14 (2 H, dd, J = 8, 14 Hz, CH_ACH_BPh), 2.14 (2 H, m, C*H*Me_2), 1.05 (12 H, d, J = 7 Hz, Me); ^{13}C NMR (75 MHz, CD_3OD) δ: 173.5, 172.1, 170.1, 157.6, 135.4, 130.5, 130.0, 128.8, 60.9, 55.3, 43.3, 42.1, 39.2, 38.5, 31.6, 19.5, 18.8; MS (electrospray) 752.3 $(M+H)^+$.

(*Received in UK* 7 *May* 1996; *accepted* 31 *May* 1996)