Pergamon

Tetrahedron Letters 41 (2000) 1553–1557

TETRAHEDRON LETTERS

Solid phase synthesis of oligoureas using *O*-succinimidyl-(9*H*-fluoren-9-ylmethoxycarbonylamino)ethylcarbamate derivatives as activated monomers

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Received 22 November 1999; accepted 15 December 1999

Abstract

An efficient stepwise synthesis of oligoureas (up to the nonamer) on solid support using *O*-succinimidyl-(9*H*-fluoren-9-ylmethoxycarbonylamino)ethylcarbamate derivatives as activated monomers is described. These building blocks were readily prepared starting from *N*-Fmoc-protected β^3-amino acids via Curtius rearrangement of the corresponding acyl azides and treatment of the resulting isocyanate with *N*-hydroxysuccinimide.

Keywords: oligomers; ureas; solid-phase synthesis; carbamates.

In the field of peptidomimetic chemistry, the creation of novel oligomeric compounds with defined secondary structures and/or biological activities has recently attracted considerable attention.[1–5] Oligoureas as peptide backbone mimetics were first described by Burgess and co-workers in 1995.[6a] The expected increased resistance to enzymatic degradation as compared to peptides, as well as hydrogen bonding properties of the urea backbone make this class of compounds particularly suitable for drug discovery and for the search of novel folded structures. With this respect, Nowick and co-workers have demonstrated in an elegant series of studies, that oligoureas can serve as molecular scaffolds for the construction of artificial β-sheets.[7] The pharmacological potential of oligoureas has been addressed only in two studies so far.[6b,8] In one of these, an HIV-1 Tat derived oligourea was synthesized and found to bind with a high affinity to the *trans*-activation responsive region (TAR) RNA.[8]

The development of efficient solid phase synthesis methods for the preparation of oligoureas is a prerequisite for rapid evaluation of potentially active compounds. Several approaches utilizing different activated monomers have appeared recently in the literature.[6,9–11] Burgess and co-workers developed

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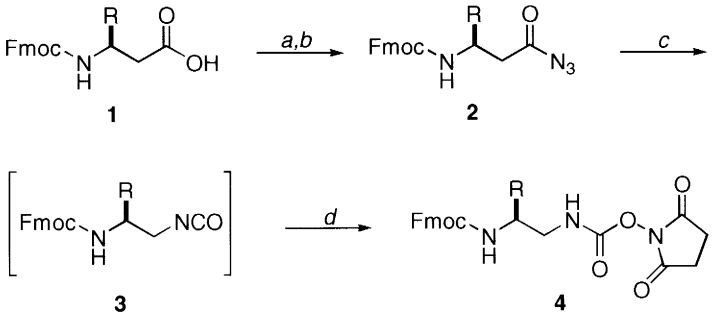
0040-4039/00/$ - see front matter

PII: S0040-4039(99)02353-9

optically pure phtalimide protected isocyanates by treatment of corresponding diaminoethane derivatives with phosgene.[6] Alternatively, optically pure azido-4-nitrophenyl carbamate monomers and *N*-Boc protected 4-nitrophenyl carbamate derivatives, prepared by reaction of the corresponding amines with 4-nitrophenyl chloroformate, have been reported.[9,10] However, *N*-Fmoc protected monomers which would represent invaluable buildings blocks for automated solid phase synthesis of oligoureas have not been reported so far. We have recently described an efficient preparation of *O*-succinimidyl-2-*(tert*-butoxycarbonylamino*)*ethylcarbamate derivatives from *N*-Boc-protected β-amino acids and their application in the synthesis of substituted ureas and oligoureas in solution.[11]

Herein, we wish to report the extension of this method to the synthesis of the corresponding *O*-succinimidyl-(9*H*-fluoren-9-ylmethoxycarbonylamino)ethylcarbamate derivatives **4** (Scheme 1).



Scheme 1. (a) EtOCOCl, NMM, THF, −15°C, 15 min; (b) NaN_3, H_2O, 5 min; (c) toluene, 65°C; (d) *N*-hydroxysuccinimide, pyridine, 65°C, 5 min

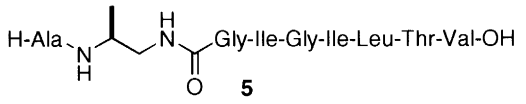
According to Scheme 1, *N*-Fmoc-protected β-amino acids (Fmoc-β^3-HXaa-OH) **1** bearing side chains of Ala, Val, Leu, Phe, Tyr, and Lys were first converted to the corresponding acyl azides **2** by reaction of their mixed anhydrides (formed with EtOCOCl/*N*-methyl morpholine (NMM)) with an aqueous solution of NaN_3 (2.5 equiv).[12,13] Intermediate isocyanates **3** obtained by Curtius rearrangement of **2** (toluene, 65°C, 5 to 15 min) were immediately trapped with *N*-hydroxysuccinimide (1 equiv.) in the presence of pyridine (1 equiv.) to afford the corresponding carbamates **4** in moderate to good yields as stable crystalline products (Table 1).[14,15] It is worth mentioning that the reaction sequence from **1** was generally complete in less than 1 h. As previously found for the corresponding Boc derivatives,[11] carbamates **4** usually precipitated or crystallized directly from the toluene solution (either hot or upon cooling) and were simply collected by filtration and washed with toluene. However, in the case of *N*-Fmoc-protected derivatives **4**, the yields were consistently better. In addition, carbamates **4** can be stored for a prolonged period of time at 4°C or even at rt without noticeable degradation.

With monomers **4a**–**f** at hand, our next endeavour was the solid phase synthesis of ureidopeptide **5** and oligoureas **6**–**8** containing six to nine urea bonds. The synthesis of **5** was achieved by coupling **4a** (4 equiv.) to the free amino group of the resin bound peptide in DMF (2 ml) in the presence of disopropylethylamine (DIEA, 10 equiv.) for 2×90 min. At the end of that time the Kaiser nihydrin test[16] was negative. The Fmoc group was deprotected with 20% piperidine in DMF and the last amino acid was introduced using standard peptide synthesis procedure.

Table 1
Conversion of β-amino acids **1** to the corresponding *O*-succinimidyl carbamates **4**

R =	carbamate **4**	Yield (%)[a]	Mp (°C)	$[\alpha]^{25}_D$ (*c*, DMF)	HPLC t_R (min)[b]
Me	**4a**	86	161-163	– 3.6 (*c* = 1.08)	10.44
*i*Pr	**4b**	69	109-111	+ 5.9 (*c* = 1.18)	11.84
*i*Bu	**4c**	51	134-137	– 10.8 (*c* = 1.01)	12.63
Bn	**4d**	66	175-177	– 26.1 (*c* = 1.13)	12.48
Bn(O*t*Bu)	**4e**	78	138-140	– 22.9 (*c* = 1.12)	13.87
$(CH_2)_4$NH(Boc)	**4f**	79	122-124	– 4.7 (*c* = 1.16)	12.67

[a]Isolated yield from **1** after crystallisation from toluene (not optimized). [b]Linear gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN containing 0.08% TFA), 30-100% B, 20 min.



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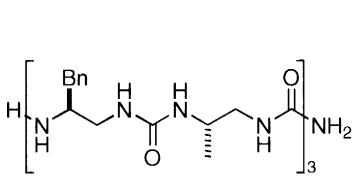

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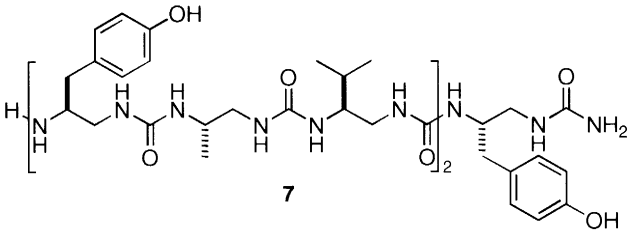

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H-β^3-HTyr^u-β^3-HLys^u -β^3-HLeu^u-β^3-HVal^u-β^3-HPhe^u-β^3-HLys^u-β^3-HAla^u-β^3-HVal^u- β^3-HTyr^u-NH_2

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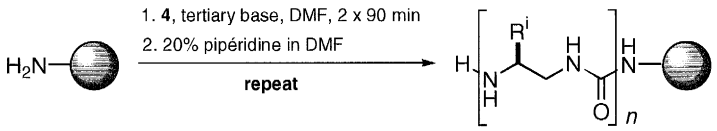
The general reaction sequence for the synthesis of oligoureas **6–8** on solid support is outlined in Scheme 2.[17] Solid phase synthesis of oligourea **6–8** was performed on Rink amide resin[18] (0.60 mmol/g; 50 μmol scale) by coupling *O*-succinimidyl carbamate **4** (4 equiv.) with DIEA (10 equiv.) in DMF for 2×90 min.[19] The Fmoc group was removed using standard conditions (3×5 min) with 20% piperidine in DMF.



Scheme 2. General procedure for the solid phase synthesis of oligoureas using *O*-succinimidyl carbamates **4**

After removal of the last Fmoc protecting group, the resin was washed and dried prior treatment with TFA:H_2O (95:5) for 2 h. Concentration in vacuo, dilution with H_2O/MeCN and lyophilization afforded the crude **5–8** in excellent yields. Purities of the crude products are given in Table 2. HPLC purification on a C_{18} column and lyophilization afforded pure **5–8** in 20–50% overall yield (entries 1–4).

The amount of impurities was found to increase along with the size of the oligomer. MALDI-TOF analysis revealed that major impurities isolated by RP-HPLC purification of crude **7** and **8** on a C_{18} column either corresponded to deletion products (that could have arisen from incomplete coupling or incomplete Fmoc deprotection in the last steps of the synthesis) or more surprisingly resulted from double insertion of some monomers. We then investigated the stability of carbamate derivatives **4** and

Table 2
Characterization of ureidopeptide **5** and oligoureas **6–8**

Entry	Compound	Base	HPLC purity of crude product (%)	Overall Yield (%)[a]	HPLC t_R (min)[b]	MALDI-TOF MS
1	**5**	DIEA	73	50	12.57[c]	842.9 $[M+H]^+$
2	**6**	DIEA	63	42	10.86[d]	846.8 $[M+H]^+$
3	**7**	DIEA	51	38	14.58[c]	1051.5 $[M+H]^+$
4	**8**	DIEA	35	20	15.14[c]	1393.0 $[M+H]^+$
5	**7**	NMM	66	57	14.70[c]	1073.2 $[M+Na]^+$
6	**7**	-	61	55	14.59[c]	1072.8 $[M+Na]^+$

[a]After RP-HPLC purification and lyophilisation. [b]Linear gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN containing 0.08% TFA). [c]5-65% B, 20 min. [d]20-80% B, 20 min.

found that significant decomposition and Fmoc deprotection occurred in DMF containing 5% DIEA (conditions close to coupling conditions). Based on these results, we investigated milder conditions for coupling procedures. On one hand, the use of a weaker base such as NMM (2 equiv.) led to significant improvement (entry 5). On the other hand, we found that the addition of a tertiary base was not mandatory since satisfactory results were obtained in the absence of base under identical reaction times (entry 6).

In summary, we have described an efficient preparation of optically active *O*-succinimidyl-(9*H*-fluoren-9-ylmethoxycarbonylamino)ethylcarbamate derivatives and their use as activated monomers in the solid phase synthesis of oligoureas up to the nonamer. We expect that this approach will enable rapid synthesis of oligourea libraries, and may facilitate the discovery of novel biologically active oligoureas.

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13. We use the previously proposed nomenclature H-β^3-HXaa-OH for the β-amino acids bearing the side chain in 3-position.[12]
14. Compound **4a**. Yield 86%. White solid; mp 161–163°C; $[\alpha]_D^{25}$ −3.5 (*c* 1.08, DMF); HPLC t_R 10.44 min (linear gradient, 30–100% B, 20 min); ^{1}H NMR (200 MHz, DMSO-d6) δ 1.03 (d, *J*=7 Hz, 3H), 2.76 (s, 4H), 2.99–3.18 (m, 2H), 3.46–3.69 (m, 1H), 4.18–4.34 (m, 3H), 7.22–7.45 (m, 4H), 7.70 (d, *J*=7 Hz, 2H), 7.89 (d, *J*=7 Hz, 2H), 8.33 (t, *J*=6 Hz, 1H). ^{13}C NMR

(50 MHz, DMSO-d6) δ 17.8, 25.2, 45.8, 46.2, 46.7, 65.2, 120.0, 125.1, 127.0, 127.5, 140.7, 143.8, 143.9, 152.1, 155.4, 170.7. MS (MALDI-TOF) *m/z* 476 $[M+K]^+$, 460 $[M+Na]^+$.

15. Compound **4c**. Yield 51%. White solid; mp 134–137°C; $[\alpha]_D^{25}$ −11 (*c* 1.01, DMF); HPLC t_R 12.63 min (linear gradient, 30–100% B, 20 min); ^{1}H NMR (200 MHz, DMSO-d6) δ 0.80 (d, *J*=7 Hz, 3H), 0.83 (d, *J*=7 Hz, 3H), 1.14–1.33 (m, 2H), 1.50–1.54 (m, 1H), 2.57 (s, 4H), 3.04–3.07 (m, 2H), 3.51–3.58 (m, 1H), 4.45–4.44 (m, 3H), 7.10 (d, *J*=8 Hz, 1H), 7.25–7.45 (m, 4H), 7.67 (d, *J*=7 Hz, 2H), 7.86 (d, *J*=7 Hz, 2H), 8.27 (t, *J*=6 Hz, 1H). ^{13}C NMR (50 MHz, DMSO-d6) δ 21.6, 23.3, 24.1, 25.2, 45.4, 46.8, 48.5, 65.1, 120.0, 125.1, 126.9, 127.5, 140.7, 143.7, 144.0, 152.0, 155.7, 170.7. MS (MALDI-TOF) *m/z* 518 $[M+K]^+$, 502 $[M+Na]^+$.
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19. Anchoring of the first monomer to the resin was monitored with ninhydrin test. However, as the chain was growing, the trinitrobenzenesulfonic acid test was found to be more reliable.