Pergamon

Tetrahedron Letters, Vol. 38, No. 41, pp. 7163-7166, 1997
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0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01762-0

Solid-Phase Synthesis of Unnatural Amino Acids using Unactivated Alkyl Halides

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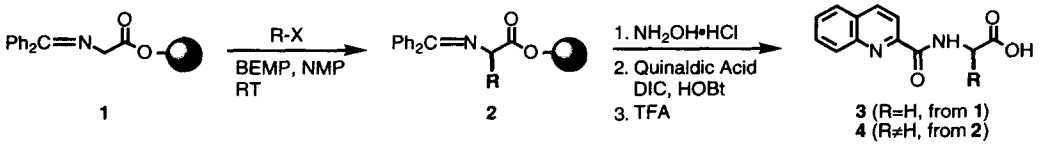
Abstract: Conditions were developed for the efficient alkylation of the resin-bound benzophenone imine of glycine with a variety of unreactive alkyl halides. Alkylations were accomplished at room temperature in NMP using the phosphazene-type base, BEMP. © 1997 Elsevier Science Ltd.

Recent work in our laboratories has focused on the solid-phase synthesis of unnatural amino acids and peptides, termed Unnatural Peptide Synthesis (UPS).[2] This methodology involves introduction of an unnatural amino acid side chain *during* a normal Solid-Phase Peptide Synthesis (SPPS) through three additional steps (activation, deprotonation/alkylation and hydrolysis). Initial studies concentrated on the use of active alkyl halides (benzylic, allylic and propargylic). UPS would allow the rapid, room-temperature introduction of many new and interesting types of amino acid side chains onto growing peptide chains if a variety of alkyl halides could be adapted to this chemistry. This study reports the use of several types of less-active halides as well as halides that can readily undergo β-elimination in competition with alkylation.[3]

To simplify the study and focus attention on the key issues involved in the alkylations, the benzophenone imine of Wang-resin bound glycine (**1**) was chosen as the starting substrate. The bulk synthesis of **1**, which is stable at room temperature for at least six months, was readily accomplished.[4,5] Standard conditions[6] for the alkylation study involved mixing the substrate (**1**) and equimolar amounts of the alkyl halide and base (2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine, BEMP) in 1-methyl-2-pyrrolidinone (NMP) and allowing the reaction to proceed at room temperature for 24 hours. The Schiff base of the resin-bound alkylated product was then hydrolyzed and the resulting free amino group condensed with quinaldic acid to give a UV active derivative, which was cleaved from the resin with TFA. The resulting derivatized product (**4**) and any unalkylated (derivatized) starting material (**3**) were then analyzed by HPLC and LC/MS.



Key variables to be addressed in the alkylation study included: different leaving groups (I, Br, Cl), the possibility of *in-situ* conversion of a poorer leaving group into a better one, and stoichiometry of the alkyl halide and base. Stoichiometry is easily varied in solid-phase reactions since excesses of soluble reagents, such as alkyl halide and/or base, are readily removed from the resin-bound product by simple filtration. Furthermore, since the UPS alkylation, like a typical phase-transfer alkylation,[7] is accomplished in the presence of *both* base and alkyl halide, it is possible to have equimolar amounts of these reagents in excess. This drives the resin-bound alkylation reaction to completion, even while a competing elimination reaction may be occurring in the solution phase.

Benzyl and n-octyl halides were used as models for active and nonactive halides, respectively, to optimize the alkylation conditions (Table 1). As reported earlier, benzyl bromide (entry 1) gives excellent results in the UPS-type alkylation with two equivalents each of alkyl halide and base.[2] However, using the same conditions, the less reactive (and less expensive) benzyl chloride (entry 2) resulted in incomplete alkylation and by-products. The results were improved by increasing the stoichiometry of the alkyl halide/base (entry 3). The best results (entry 4) were obtained by using an excess of benzyl chloride together with two equivalents of tetrabutylammonium iodide, which likely accomplishes an *in-situ* conversion of benzyl chloride to the more reactive iodide.[8]

Table 1. Alkylation of Resin-Bound Glycine Schiff Base **1** with Alkyl Halides.[a]

Entry	Alkyl halide	RX (equiv)	BEMP (equiv)	Bu_4NI (equiv)	% Mass Recovery	% 3[b] "SM"	% 4[b] "Prod"
1	$PhCH_2Br$ (model)[2]	2	2	-	84	0	100
2	$PhCH_2Cl$	2	2	-	53	8	59
3	"	10	10	-	71	1	87
4	"	2	2	2	81	0	95
5	n-$C_8H_{17}I$	2	2	-	52	0	73
6	"	10	10	-	86	0	92
7	n-$C_8H_{17}Br$	2	2	-	50	0	74
8	"	10	10	-	50	1	76
9	"	2	2	2	39	1	80
10	"	10	10	10	68	0	95
11	n-$C_8H_{17}Cl$	2	2	-	48	67	0
12	"	10	10	-	55	58	3
13	"	2	2	2	54	66	2
14	"	10	10	10	55	10	71

[a]Bold entries represent the best current conditions. [b]HPLC quantitation (220 nm).

The less reactive n-octyl halides required more extensive study (Table 1). While use of the standard conditions with n-octyl iodide (entry 5) consumed all starting material, the purity of product was only moderate because of the presence of several minor impurities. The product yield and purity were improved substantially by increasing the amount of alkyl halide/base (entry 6). With n-octyl bromide both increased stoichiometry and added tetrabutylammonium iodide were needed to realize a good yield and purity of product (entry 10) and with

n-octyl chloride (entries 11-14) it was not possible to completely consume the starting material, even by using 10 equivalents of both alkyl halide and quaternary ammonium iodide (entry 14).

With the results of these model studies in hand, attention was turned to using a variety of alkyl halides in the UPS process (Table 2). Methyl iodide, an alkyl halide of intermediate reactivity, gave excellent results using the standard conditions (entry 15).[9] Alkyl halides prone to β-elimination (entries 16-18) gave excellent yields of product as long as equimolar excesses of both the alkyl halide and the base were used.[10] Likewise, sterically demanding alkyl halides, such as isopropyl iodide (2° halide) and isobutyl iodide (1° halide containing a β-branch) also gave good yields of product (entries 19 and 20) as did a relatively unreactive β-silyloxy bromide (entry 21). Finally, alkylation with cyclohexyl iodide (2° halide susceptible to β-elimination) gave a 77% yield of product with little glycine present (entry 22).[11]

Table 2. Alkylation of **1** with Various Non-Active Alkyl Halides.

Entry	Alkyl halide	RX (equiv)	BEMP (equiv)	Bu_4NI (equiv)	% Mass Recovery	% **3**[a] "SM"	% **4**[a] "Prod"
15	CH_3I	2	2	-	87	0	97
16	CH_2=$CHCH_2CH_2Br$	10	10	-	74	0	100
17	$PhCH_2CH_2Br$	10	10	-	80	0	100
18	$PhCH(CH_3)Br$	10	10	-	87	0	95†
19	$(CH_3)_2CHI$	10	10	-	73	0	93
20	$(CH_3)_2CHCH_2I$	10	10	-	68	1	87
21	$BrCH_2CH_2OTBDMS$	10	10	10	78‡	0	91
22	c-$C_6H_{11}I$	10	10	-	70	4	77

[a]HPLC quantitation (220 nm). †Diastereomers observed. ‡Product is homoserine lactone.

In conclusion, we have developed mild conditions for the solid phase synthesis, using unreactive alkyl halides, of several novel types of amino acids. Under appropriate conditions benzylic bromides or chlorides, methyl iodide, 1° or 2° aliphatic iodides or bromides and alkyl halides that are prone to β-elimination are effective in these reactions. This UPS methodology is being extended to the dialkylation of resin-bound amino acid derivatives[12] as well as the mono- and dialkylation of peptides.

Acknowledgment. We gratefully acknowledge the National Institutes of Health (GM28193) and the Lilly Research Laboratories for support of this research.

REFERENCES AND NOTES

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4. Preparation of **1**: All reactions were run at ambient temperature. Fmoc-Gly-Wang (23.0 g, 12.6 mmol, 0.55 mmol/g, Novabiochem) was suspended in 150 mL 30% piperidine/NMP and the resulting slurry was mixed by rocking for 30 min. The resin was filtered and washed with NMP (5 x 150 mL). Benzophenone imine (22.95 g, 126 mmol, 10 equiv) in 150 mL NMP was added to the resin followed by glacial HOAc (6.6 g, 110 mmol, 8.7 equiv) and the suspension was rocked for 16 h. The resin was filtered and washed with 5 x 150 mL each of NMP and CH_2Cl_2 and dried *in vacuo* at 45°C to afford 22.6 g (0.56 mmol/g.) This resin was stored in an amber glass vial under nitrogen.
5. Initial studies (reference 2) for formation of benzophenone imines of resin-bound amines used 1.5 equiv of benzophenone imine (Merrifield resin) or 1.5 equiv of benzophenone imine and 1.3 equiv of HOAc (Wang resin). We have found the use of the larger excess of reagents guarantees complete conversion to the imine.
6. Typical reaction procedure: All reactions were run at ambient temperature.
   1 to **2**: 0.175 g of **1** (0.10 mmol) was suspended in 0.85 mL NMP and 1.33 M 1-iodooctane/NMP (0.15 mL, 2 equiv) followed by 0.5 mL of 0.4 M BEMP/NMP (2 equiv) and the reaction mixture was mixed for 24 h. The resin was filtered and washed with 3 x 3 mL each of DMF, CH_2Cl_2, THF, and THF/H_2O (3:1) to give **2**. For reactions involving nBu_4NI, 0.25 mL of 0.35 M nBu_4NI/NMP (2 equiv) was added prior to the base. When adding 10 equiv of these reagents, only 0.25 mL NMP was added followed by 0.75 mL of the R-X solution, 0.5 mL of a 2 M BEMP/NMP solution, and the nBu_4NI was added as a solid due to low solubility.
   2 to **4**: The alkylated resin **2** was suspended in 1M NH_2OH•HCl/THF (3:7 v/v) and mixed for 5 h. The reaction mixture was filtered and washed with DMF (5x3 mL), 10% DIEA/NMP (3x3 mL), and DMF (10x3 mL). 1.0 M Quinaldic acid/NMP (0.5 mL, 0.5 mmol) and 1.0 M 1-hydroxybenzotriazole/NMP (0.5 mL, 0.5 mmol) were added to the resin. Diisopropyl carbodiimide (78 µL, 0.5 mmol) was then added and the reaction mixture was mixed for 18 h. The reaction mixture was filtered and washed with 5x3 mL each of DMF, CH_2Cl_2/MeOH (1:1), MeOH, and CH_2Cl_2. This resin was suspended in 95% TFA/water (2.0 mL) and mixed for 4 h. The aqueous TFA was then filtered into a tared vial and the resin was washed with 95% TFA/water (2x0.5 mL) and added to the filtrate. This solution was concentrated under a flow of nitrogen at 50 °C and the residue was then dried *in vacuo* to yield crude **4** as a red oil that solidifies on standing. Yields are for the unpurified products. Quantities of **3** and **4** were determined by HPLC using a Vydac Protein and Peptide C18 column (150 x 4.6 mm) with mobile phases consisting of 0.1% (v/v) TFA/H_2O (A) and 0.08% (v/v) TFA/CH_3CN (B) with a gradient of 0 - 80 % B in 20 min at a flow rate of 1 mL/min with detection at 220 nm.
7. For lead references concerning phase-transfer alkylations, see: *Phase-Transfer Catalysis, Mechanisms and Synthesis*, ACS Symposium Series: 659, Halpern, M., Ed., American Chemical Society: Washington, D.C., 1997.
8. Lead reference concerning the Finkelstein reaction (halide exchange): Landini, D.; Albanese, D.; Mottadelli, S.; Penso, M. *J. Chem. Soc., Perkin Trans 1*, **1992**, 2309-2311. Use of catalytic amounts of nBu_4NI did not improve yields significantly.
9. Under conditions identical with those used for MeI: Me_2SO_4 gave 10% **3** and 59% **4** while MeOTf resulted in 57% **3** and a trace of product **4** together with several impurities.
10. For comparison, alkylation with n-octyl iodide (10 equiv) and BEMP (2 equiv) resulted in complete conversion (0% **3**, 90% **4**). In contrast, with phenethyl bromide the same amounts of alkyl halide (10 equiv) and BEMP (2 equiv) gave incomplete conversion of starting material and several impurities (7% **3**, 57% **4**). In the latter case, the base (2 equiv) is likely used up in an elimination reaction with the phenethyl bromide before complete deprotonation of the substrate **1** has occurred.
11. When the alkyl halides, cyclohexyl iodide and 2-iodopropane, were prepared by an *in situ* Finkelstein reaction on the corresponding bromides yields were lower.
12. For the solid-phase synthesis of α,α-disubstituted unnatural amino acids and peptides (di-UPS), see: Scott, W. L.; Zhou, C.; Fang, Z.; O'Donnell, M. J. *Tetrahedron Lett.*, **1997**, *38*, 3695-3698.

(*Received in USA* 16 *June* 1997; *accepted* 18 *August* 1997)