Study of the Racemization Observed in the Amide Bond Forming Reaction on Silica Gel

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Abstract

Racemization resulting from the coupling of N(3,5-dinitrobenzoyl)-L-leucine and 3-aminopropyl silica gel with several amide-coupling reagents is further investigated in order to explain the much higher degree of racemization on silica gel, as compared with the similar reaction in solution. Based on experiments using different types of solid supports, limited pore access and surface microchemical environment are ruled out as the possible reason for the higher degree of racemization that occurred on silica gel. Steric hindrance of the solid support is thought to have caused the amino group to be more basic relative to its nucleophilicity, leading to a higher degree of racemization.

Introduction

One of the popular methods to immobilize stationary phases onto silica gel involves amide bond formation (1,2). In this approach, stationary phases derivatized with a free carboxylic acid group are coupled to silica gel derivatized with an amino group (typically 3-aminopropylsilica gel) by means of a proper coupling reagent. The advantage of this method is that stationary phases with a free carboxylic acid group, as well as silica gel derivatized with an amino group, are stable and often easily prepared. In this reaction, racemization may occur if chiral reactants are involved. In a previous publication, the degree of racemization that occurred when a modified amino acid was coupled to 3-aminopropylsilica gel was determined (3). A large degree of racemization was observed in this amide bond-forming reaction on silica gel using several common coupling reagents. In contrast, similar reactions in solution yielded a much lower degree of racemization.

Initially, it was thought that restricted access to pore structures was responsible for the enhanced degree of racemization on silica gel. In this paper, evidence is presented that contradicts such an explanation. This evidence includes racemization studies on silica gel with lower surface ligand concentration, on silica gel with larger pore sizes, and on Rink amide resin (a popular peptide synthesis resin). A new explanation is proposed based on these results.

Experimental

General supplies and equipment

N-(3,5-dinitrobenzoyl)-L-leucine (DNB-L-Leu-OH), all other chemicals, and solvents were purchased from either Aldrich (St. Louis, MO), Fluka (Ronkonkoma, NY), or Fisher Scientific (Pittsburgh, PA) unless otherwise noted. High-performance liquid chromatography (HPLC)-grade Allsphere silica gels (5-µm particle size, 80-Å pore size, and 220-m^2/g surface area; 5-µm particle size, 300-Å pore size, and 100-m^2/g surface area) were purchased from Alltech (Deerfield, IL). Rink amide resin in, its 9-fluorenylmethoxycarbonyl (Fmoc) form (100–200 mesh, 0.74-mmol/g amino loading, 1% divinylbenzene), was purchased from NovaBiochem (La Jolla, CA). Selecto silica gel (32–63 µm) from Fisher Scientific was used for flash column chromatographic purification of target compounds. Thin-layer chromatography (TLC) was completed using EM silica gel 60 F-254 TLC plates (0.25 mm) (Merck, Darmstadt, Germany). An S-N1N-naphthylleucine column was purchased from Regis Technologies (Morton Grove, IL). Elemental analyses were conducted by Atlantic Microlab, Inc. (Norcross, Georgia). ^{1}H NMR spectra were recorded with a Bruker 300 MHz instrument (Billerica, MA). HPLC analyses were carried out on a System Gold analytical gradient system (Beckman Coulter, Fullterton, CA).

Preparation of 3-aminopropyl silica gel (80 Å) with low surface loading

One gram of 80-Å silica gel, previously acid washed with nitric and sulfuric acids, was refluxed with 20 µL of 3-aminopropyltriethoxysilane in 10 mL of toluene overnight to produce 3-aminopropyl silica gel. Surface coverage was determined by elemental analysis to be 0.09 mmol/g based on percent N (elemental analysis: C, 1.26; N, 0.12; and H, 0.56). For comparison, 3-aminopropylsilica gel prepared using normal amount of 3-aminopropyltriethoxysilane has a surface coverage of 0.48 mmol/g (4).

Preparation of 3-aminopropyl silica gel (300 Å)

This material was prepared according to the published procedure (3). Surface coverage was determined by elemental analysis to be 0.22 mmol/g based on percent N (elemental analysis: C, 1.13; N, 0.31; and H, 0.24).

Preparation of 4-aminobutyric acid functionalized rink amide resin (4-aminobutyric acid-Rink resin)
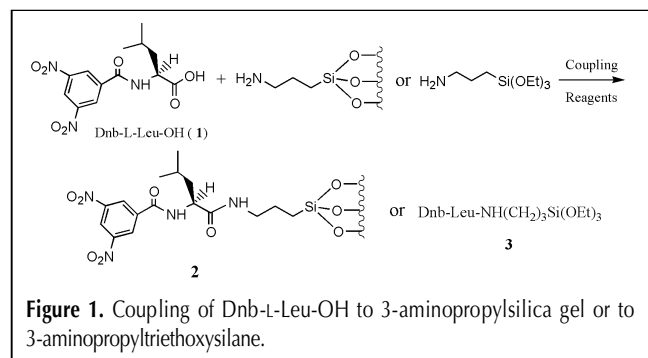
Rink amide resin (1.5 g) in its Fmoc form, preswelled with dichloromethane (DCM), was modified by removal of the Fmoc group with 20% piperidine in N,N-dimethylformamide (DMF) (10 mL) by shaking for 30 min. Fmoc-4-aminobutyric acid (Abu)-OH (2 equiv) was coupled onto 1.5 g of the deprotected resin with benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluorophosphate (PyBop) (3 equiv), 1-hydroxybenzotriazole (HOBt) (1 equiv), and N,N-diisopropylethylamine (DIPEA) (4 equiv) for 3 h with shaking. Thereafter, the Fmoc-protecting group was removed from the resin with 20% piperidine in DMF 20% (10 mL) by shaking for 30 min.

Coupling of Dnb-L-Leu-OH to amino groups on different solid supports

The following coupling reagents were used individually to couple Dnb-L-Leu-OH onto modified Rink amide resin: 80 Å aminopropylsilica gel with a decreased loading of aminopropyl groups and 300 Å aminopropylsilica gel; diisopropylcarbodiimide (DIC)-HOBt; DIC-1-hydroxy-7-azabenzotriazole (HOAt); diphenylphosphoryl azide (DPPA) with DIPEA; 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ); pentafluoro-phenyl diphenylphosphinate (FDPP) with DIPEA; O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) with DIPEA; and PyBop with DIPEA (2 equiv coupling reagents with respect to amino groups on the solid support). All reactions were done in 2 mL of 20% DMF–DCM for 6 h with shaking using 100 mg solid support.

Determination of the degree of racemization for the coupling reaction on Rink amide resin

The Dnb-Leu-Abu was cleaved from the resin as Dnb-Leu-Abu-NH$_2$ by treatment with 95% trifluoroacetic acid–DCM for 30 min. After the reaction, the supernatant containing Dnb-Leu-Abu-NH$_2$ was rinsed into a round-bottom flask with MeOH and DCM. After concentration, the crude sample was passed through a plug of silica gel with 20% MeOH–DCM. The solvents were evaporated and the enantiomeric purity of the resulting Dnb-Leu-Abu-NH$_2$ obtained was determined by HPLC using the S-N1N-naphthylleucine column [mobile phase: methanol–CH$_2$Cl$_2$ (85/15); UV detection was at 254 nm; flow rate 1.2 mL/min]. Percent racemization was calculated based on the difference in the peak area of the enantiomers.

Determination of the degree of racemization for the coupling reactions on silica gel

The degree of racemization on silica gel was determined according to the procedure published previously (4).

Results and Discussion

The system studied in the previous paper is the coupling reaction between dinitrobenzoylleucine (Dnb-L-Leu-OH) and 3-aminpropylsilica gel (Figure 1) (3). In that study, seven amide-coupling reagents were investigated. For control experiments, the coupling between Dnb-L-Leu and 3-aminopropyltriethoxysilane (the solution phase coupling), a good model for 3-aminopropylsilica gel (Figure 1), was studied. The results obtained for the solution phase coupling follow expectations as inferred from various literature reports (Table I) (5,6). On silica gel, however, significant amounts of racemization (the maximum amount of D-enantiomer is 50%) were observed with all except one coupling reagent (EEDQ) [Table I, column 3-Aminopropylsilica Gel (80 Å)].

The significant amount of racemization observed in the amide coupling reactions on silica gel was unexpected, considering that the same amount of racemization was not observed in solution. The mechanisms for racemization in amide bond formation in peptides are well studied, and the most likely racemization path is through the formation of an azlactone (Figure 2) (7). Once an amino acid is activated by a coupling reagent, two reaction paths become available. The activated amino acid (**4**) could react directly with the amine, leading to the formation of the desired product L-**5** without any racemization (path A).

Figure 1. Coupling of Dnb-L-Leu-OH to 3-aminopropylsilica gel or to 3-aminopropyltriethoxysilane.

Table I. Racemization Observed in the Coupling of Dnb-L-Leu-OH (1) to 3-Aminopropyltriethoxysilane (Solution), 3-Aminopropylsilica Gel (APS) (80 Å), Low Density 3-Aminopropylsilica Gel (80 Å), 3-Aminopropylsilica Gel (300 Å), or Abu-Rink Amide Resin (Rink Resin)*

	D-Enantiomer in product (%)				
Reagents	Solution	APS (80 Å)	Low density APS (80 Å)	APS (300 Å)	Rink resin
DIC–HOAt	6.2	33	42	37	16
DIC–HOBt	12	30	45	36	25
DPPA	16	34	n.a.[†]	n.a.	35
EEDQ	3.6	3.7	n.a.	5.9	5.2
FDPP	18	40	n.a.	n.a.	30
HATU	3.4	30	31	28	30
PyBop	14	40	39	38	38

* Starting material Dnb-L-Leu-OH (1) contains 2% D-enantiomer.
† n.a. = not available.

The other scenario involves an intramolecular rearrangement, leading to the formation of an azlactone (**6**) (path B). The α-proton in the azlactone (**6**) is rather acidic because of extensive conjugation. Therefore, azlactone (**6**) can be deprotonated and reprotonated, causing its racemiztion (path C). Both enantiomers of azlactone could react further with the amine, leading to their corresponding amides as the final products (paths D and E).

According to this mechanism, the extent of racemization in the product is determined by the relative rates of five reaction steps (A, B, C, D, and E). If the reaction of the amine with the azlactone or the initially formed activated intermediate is fast (steps A and D), relative to steps B and C, lower degree of racemization is expected. If steps B and C are fast relative to steps A and D, a larger degree of racemization will result.
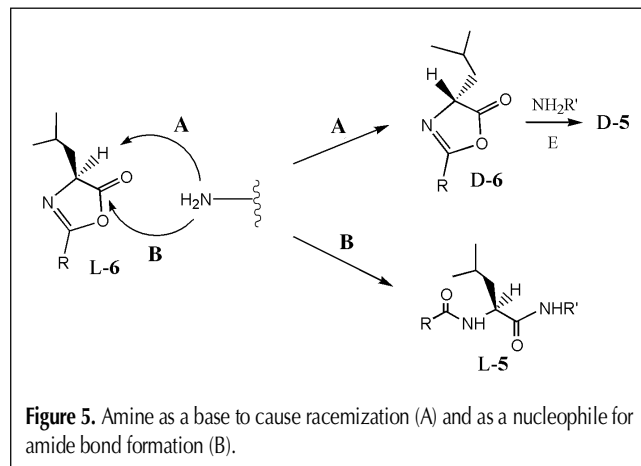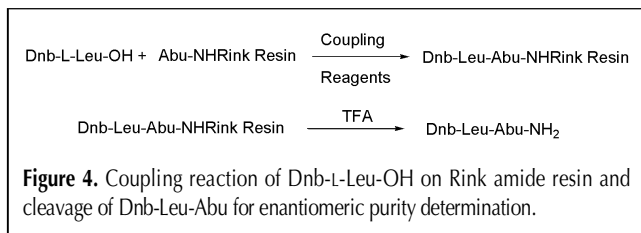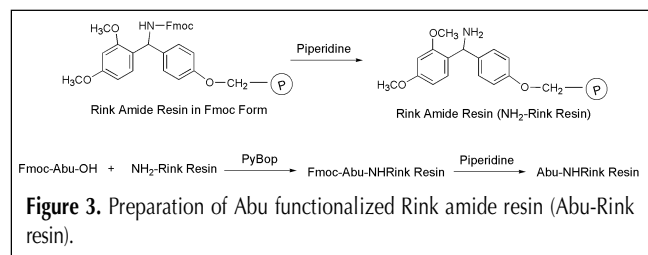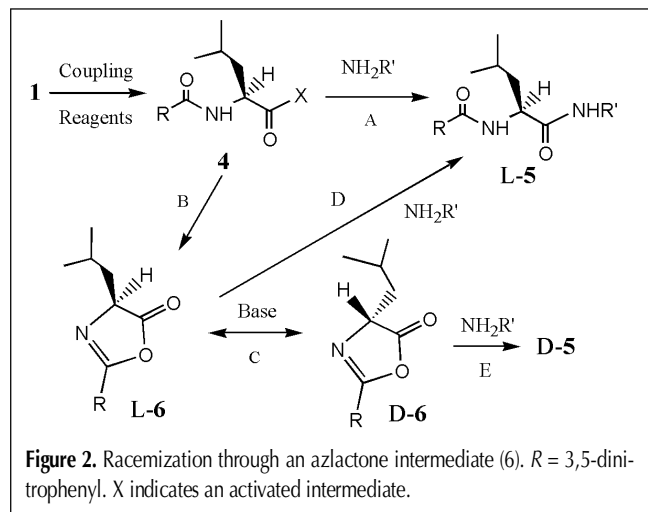
Applying this mechanism to our system, step B should have similar reaction rates in both solution reaction and on silica gel, as it is determined by the structure of the acid **1** and the coupling reagents common to both conditions. Other steps (A, C, D, and E), however, can show different reaction rates. Steps D and E should have exactly the same reaction rate.

Several factors could influence the relative rates of these individual steps and thus be responsible for the large degree of racemization observed. The first factor is the limited access to the amino groups in certain parts of the pores of silica gel. It is conceivable that such limited access could slow down step A relative to step B, causing a large degree of racemization. Two experiments were designed to verify if this limited pore access explanation is viable. In one experiment, coupling reactions were studied on silica gel with lower surface amino loading. Silica gel of this kind was prepared using a controlled amount of 3-aminopropyl triethoxysilane (see Experimental

section). Another experiment involved coupling reactions using silica gel with larger pore size (300 Å). If limited access to the amino groups in certain parts of the pores of silica gel is responsible, a lower degree of racemization should be observed on these two types of silica gel. However, as seen in Table I, degrees of racemization obtained on these two types of silica gel are comparable to those obtained previously. These results indicate that limited pore access is not responsible for the large degree of racemization observed.

The second factor considered is the microchemical environment near the surface of silica gel (8). Chemical properties of the surface of silica gel are very different from those of the organic solvents used for these racemization studies. It is possible that reactions on the surface of silica gel can be impacted by its properties. To verify whether such microchemical environment is responsible for the observed racemization, coupling reactions on Rink amide resin, a polystyrene-divinylbenzene resin commonly used for solid-phase peptide synthesis, was investigated. The surface of Rink amide resin is hydrophobic and free from silanol groups, very different from that of silica gel.

For this study, Rink amide resin was functionalized with Abu to introduce a primary amino group that is very similar to the primary amino group in 3-aminopropyl silica gel, according to Figure 3. Coupling reactions of Dnb-L-Leu-OH to this amino-functionalized Rink amide resin (Abu-Rink resin) were then studied under conditions used for silica gel experiment. After the reaction, Dnb-Leu-Abu-NH_2 was cleaved off the resin by treatment with trifluoroacetic acid (Figure 4). Degree of racemization was then measured by analyzing the enantiomeric purity of the resulting Dnb-Leu-Abu-NH_2 using a commercial chiral column (see Experimental section). It should be pointed out that acid treatment did not introduce an additional amount of racemization, otherwise a significant amount of racemization would have also been observed in EEDQ coupling. It is clear from Table I that

Figure 2. Racemization through an azlactone intermediate (6). *R* = 3,5-dinitrophenyl. X indicates an activated intermediate.

Figure 3. Preparation of Abu functionalized Rink amide resin (Abu-Rink resin).

Figure 4. Coupling reaction of Dnb-L-Leu-OH on Rink amide resin and cleavage of Dnb-Leu-Abu for enantiomeric purity determination.

Figure 5. Amine as a base to cause racemization (A) and as a nucleophile for amide bond formation (B).

the degree of racemization on this Rink amide resin is comparable to those on silica gel. Therefore, one needs to rule out microchemical environment as the reason for the large degree of racemization observed.

The steric hindrance of solid supported functional group is then considered. When a functional group is attached to a solid support, it is reasonable to assume that it becomes more sterically hindered than the same group in solution. Such steric hindrance has been proposed to explain the selectivity observed in a reaction that involved a supported crown ether reagent (9). It should be noted that the amino group in the coupling reaction, besides being the nucleophile to form the amide bond, could also act as a base to racemize the azlactone L-**6** (Figure 5). In fact, it could be mainly responsible for racemization in all the coupling reactions studied. In some of the coupling reactions, this was the only base added. In other reactions, DIPEA was also used. The hydrogen bond acceptor strength (pK_{HB}), a better measure of nonequilibrium basicity, of this sterically hindered tertiary amine has been determined. Compared with *n*-butylamine with a pK_{HB} value of 2.21, this amine has a pK_{HB} value of only 1.05 (10,11). Therefore, the primary amine should be more potent in inducing racemization.

It can be further assumed that the degree of racemization could depend on the relative basicity and nucleophilicity of the amine, with stronger basicity leading to more racemization. Steric hindrance is expected to impact nucleophilicity more than basicity, as the target of the nucleophile (a substituted carbon) is much bulkier than the target of a base (a hydrogen). The sterically hindered DIPEA supports this argument of the steric impact on the relative nucleophilicity and basicity, as it is commonly used as a non-nucleophilic base.

Accepting these arguments, the larger amount of racemization observed on solid support can be explained. Steric hindrance could slow steps D and E more than step C, resulting in a large degree of racemization. The fact that no significant racemization was observed with EEDQ in both solution and on silica gel indicates that conversion from the activated amino acid to azlactone is probably slow (Step B) compared to its direct conversion to the final product (Step A) for this coupling reagent. Because azlactone formation is required before racemization can occur, a slow step B leads to less racemization.

Conclusion

In summary, it is thought that the amino group of the 3-aminopropylsilica gel could also act as a base to cause racemization, in addition to its role as a nucleophile for amide bond formation. Steric hindrance of the solid support may cause the amino group to be more basic relative to its nucleophilicity, thus leading to a higher degree of racemization. Limited pore access and surface microchemical environment are ruled out as the possible source of the higher degree of racemization on silica gel.

Acknowledgments

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