

Norbornyl Dipeptide Analogues: Mimics of Both a Transition State and a Torsionally Distorted Ground State

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The 1-amino-2-hydroxybicyclo[2.2.1]heptane-7-carboxylic acid derivatives **1-3** have been synthesized, the pivotal step being the use of an acyl nitrene-insertion reaction to introduce nitrogen functionality into the corresponding hydroxy ester. The analogues each mimic a distorted peptide ground state as well as the transition state for peptide bond hydrolysis. To enhance the immune response and to provide further sequence specificity, the analogues **1-3** have been coupled to at least one D-amino acid residue to yield the derivatives **21**, **26**, and **27**, respectively. Antibodies elicited against these derivatives may catalyze the hydrolysis of the corresponding peptides both by straining the substrate ground state and by stabilizing the transition state. © 1995 Academic Press, Inc.

INTRODUCTION

Analogues of amino acids and peptides are being utilized with increasing frequency in the study of biological systems. Unnatural amino acids can now be efficiently incorporated into peptides and proteins (1), a methodology that has facilitated the study of key residues in both catalytic and folding processes (2). Conformationally restricted peptide analogues (3) have been used to probe the biologically active conformations of peptides (4) and to model regions of defined structure such as β -sheet (5) or α -helix (6) initiation sites. A variety of enzymological studies have utilized peptide derivatives as mechanistic probes (7), and peptide-based transition state analogues can be potent protease inhibitors (8). Finally, most efforts directed toward the isolation of antibodies with peptidase activity have utilized peptide analogues as haptens (9). It is in this latter application that we have particular interest.

Since the first reports of antibody catalysis, the field has grown rapidly and has been extensively reviewed (10). While a number of promising reports have appeared (11), no *general* method has yet been developed for the elicitation of peptidolytic antibodies. Thus, we have been pursuing a new strategy for the isolation of antibody peptidases: immunization with analogues that mimic a distorted peptide ground

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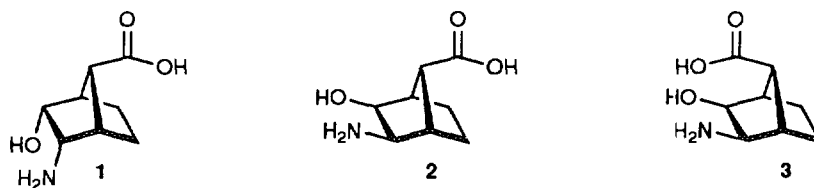
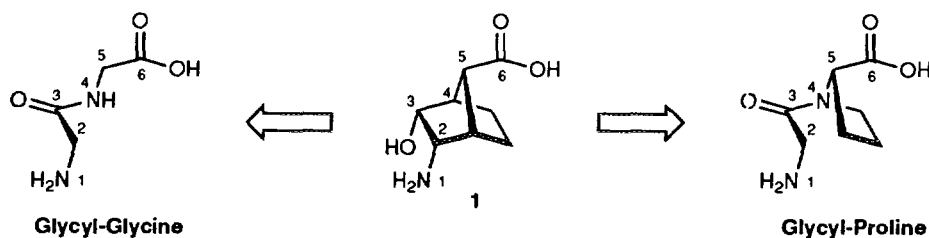


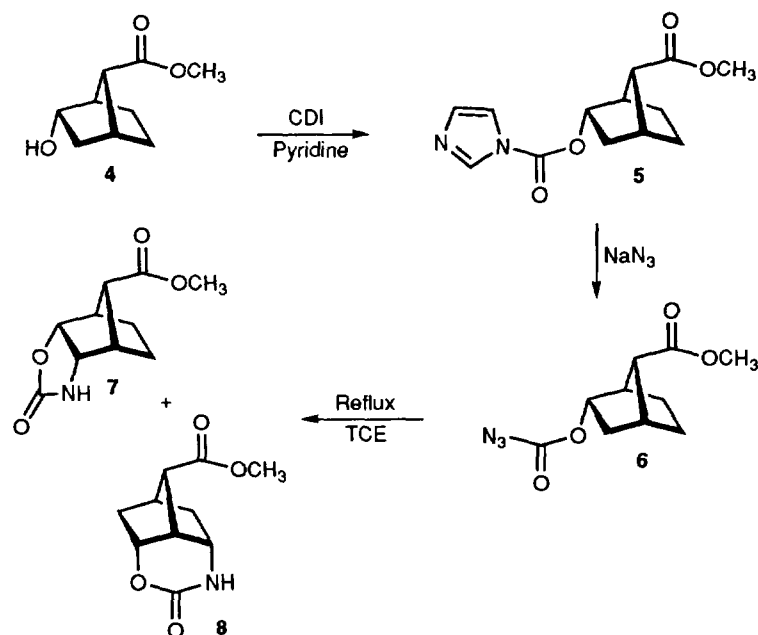
FIG. 1. Structures of the dipeptide analogues.

state in addition to the transition state for peptide bond hydrolysis. The antibodies elicited may catalyze hydrolysis of the corresponding peptide by both straining (or stressing (*12*)) the substrate ground state and by stabilizing the transition state. (Note that these two catalytic strategies are not, as has been discussed, independent (*13*.) We have described the detailed rationale for this approach elsewhere (*14*).

Here we report the synthesis of the 1-amino-2-hydroxybicyclo[2.2.1]heptane-7-carboxylic acid derivatives **1–3** (Fig. 1), each as a racemic mixture. As is shown in Fig. 2 for derivative **1**, the norbornyl framework is isosteric with both glycyl-glycine and glycyl-proline. The hydroxyethylene isostere mimics the transition state for peptide bond hydrolysis, while the norbornyl framework ensures that the peptide bond mimicked is distorted from planarity. Precedent for the use of these structural features comes from a variety of sources. Torsionally distorted amides, similar in geometry to the norbornyl derivatives here, have been shown to hydrolyze many orders of magnitude faster than their unstrained counterparts (*15*). The hydroxyethylene group has been demonstrated to be an effective transition state analogue: it is present in the pepsin inhibitor pepstatine (*16*), has been incorporated into potent HIV protease inhibitors (*17*), and has successfully been used to generate antibody catalysts, including those with esterase and imidase activity (*18, 11d*). (While tetrahedral phosphorus derivatives are arguably the best mimics of hydrolytic transition states, their incorporation into norbornyl derivatives is less than straightforward.) To enhance the immune response and to provide further sequence specificity, the analogues **1–3** have been coupled to additional amino acid residues to yield the derivatives **21, 26, and 27**, respectively (Schemes 5 and 6).

The stereoisomers **1–3** differ in the relationship of the carboxylic acid and the

FIG. 2. Alignment of glycyl-glycine and glycyl-proline with analogue **1**.



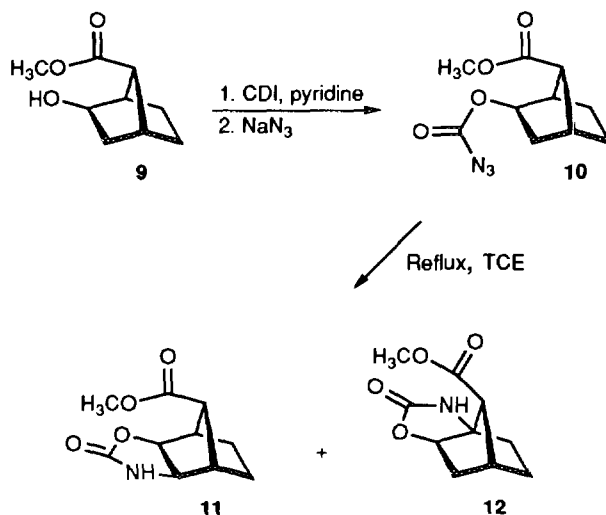
SCHEME 1

1,2-amino alcohol functionalities (*syn* versus *anti*), and in the relative orientation of the 1,2-amino alcohol functionality to the norbornyl ring (*endo* versus *exo*). The pivotal step in the preparation of these derivatives is the use of an intramolecular acyl nitrene-insertion reaction to introduce nitrogen functionality into the corresponding hydroxy methyl ester derivatives. Because of their reactive nature and the harsh conditions required for their formation, nitrenes have been used only occasionally in synthesis (19). Nonetheless, the results described here illustrate the utility of this reaction in the synthesis of dipeptide analogues. (We have also reported the use of this method to synthesize cyclobutyl and spiro[4.4]nonyl dipeptide analogues (20).)

Finally, the derivatives described here may also find applications as probes of structure–function relationships in peptides and proteins; in particular, the rigid norbornyl ring system may give access to constrained peptide geometries that currently are unavailable. When a transition-state mimic is not required, the unprotected hydroxyl group may be oxidized to a ketone, resulting in the conversion of a hydrogen-bond donor to a hydrogen-bond acceptor.

RESULTS AND DISCUSSION

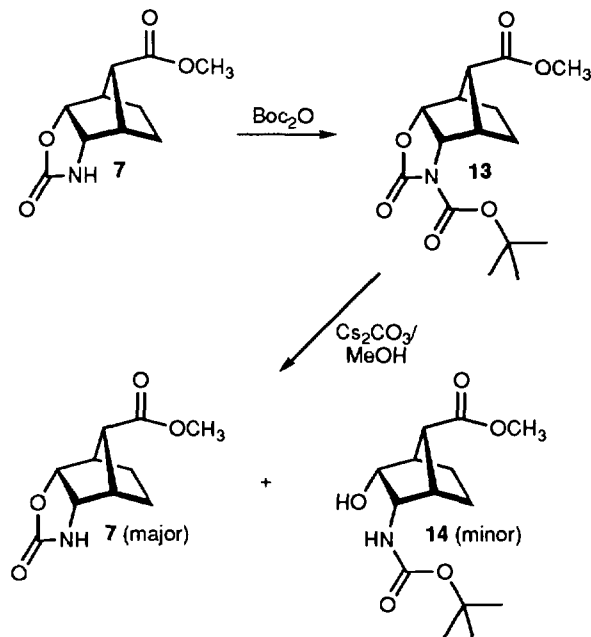
Preparation of azidocarbonate derivatives. As is outlined in Scheme 1, the norbornyl analogue 7 was synthesized, following the strategy of Lowe and Swain (21), from the corresponding hydroxy methyl ester 4 (22). The derivative 4 was treated



SCHEME 2

with carbonyldiimidazole (CDI) to give the carbonyl imidazole derivative **5**, which was reacted directly with NaN_3 to form the azidocarbonate derivative **6**. Both steps in this sequence were readily monitored by ^1H NMR, as each reaction is accompanied by a characteristic change in chemical shift of the carbinol proton: its resonance in the carbonyl imidazole derivative is approximately 0.8 ppm downfield from that in the reactant alcohol, and its resonance in the azidocarbonate derivative is approximately 0.3 ppm upfield relative to that in carbonyl imidazole derivative. Thermal decomposition, in refluxing 1,1,2,2-tetrachloroethane (TCE), of the azidocarbonate **6** yielded the cyclic carbamate **7** as the predominant product; a minor product was the six-membered cyclic carbamate **8**. In a similar fashion, the hydroxy ester precursor **9** (23), was elaborated into the azidocarbonate **10** (Scheme 2). Thermolysis yielded the two cyclic carbamates **11** and **12** in approximately equal yield. (Consistent with the geometrical constraints and with a previous study of the regiochemistry of carbene insertions in norbornyl derivatives (24), *exo*-insertion of the *endo*-azidocarbonate **6** or *endo*-insertion of the *exo*-azidocarbonate **10** was never observed.)

Thermolysis of azidocarbonates. Initial work in our laboratory on the azidocarbonate **6** and on azidocarbonates of two cyclobutyl derivatives (20a) involved thermolysis of a methylene chloride (CH_2Cl_2) solution in a sealed heavy-walled glass tube or in a Parr bomb reactor. Since dilute solutions of azide are required to avoid intermolecular insertion reactions, this method was limited in scale by the size of the commercially available apparatus. As both photolysis and thermolysis of the steroid derivative 3β -lanostenyl azidocarbonate had been reported to give the corresponding cyclic carbamate in similar yields (25), we investigated the photolysis reaction of azidocarbonate **10** as a means of increasing the yield of nitrene insertion.

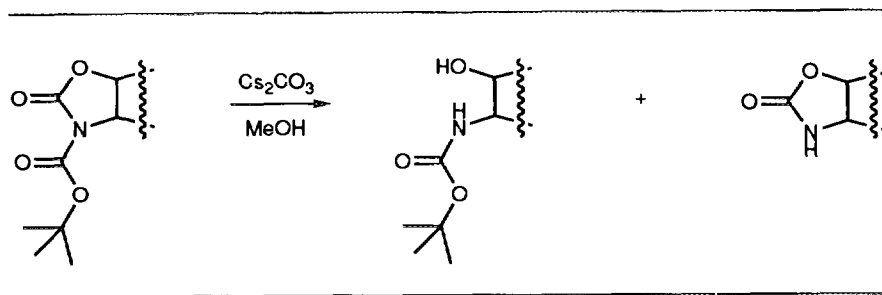
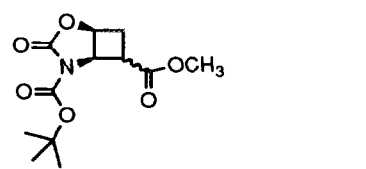
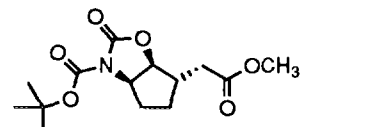
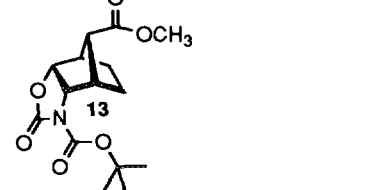
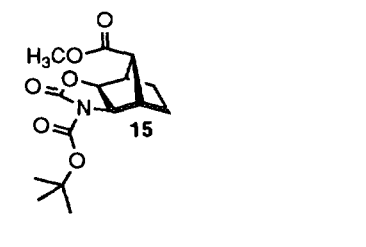


SCHEME 3

Unfortunately, the major product was the primary amino carbamate, presumably arising from proton abstraction from the solvent CH_2Cl_2 , albeit a poor hydrogen donor. None of the desired cyclic carbamate was detected. Returning to the thermolysis reaction, we found that simply refluxing a dilute solution of an azidocarbonate in TCE (**19**), which has a boiling point of 147°C , for 30–60 min gave the desired product in comparable yield to high-pressure thermolysis without the limitation of scale. While these conditions are relatively harsh, closer investigation of the thermolysis of azidocarbonate **6** showed that the reaction was complete after 10 min; this shorter reaction time may be useful in the preparation of more labile compounds.

Cleavage of the cyclic carbamates. Prior to further elaboration, the carbamate derivatives had to be cleaved. Previously, with a series of cyclobutyl dipeptide analogues (**20a**), we reported cleavage of cyclic carbamates in high yield by the method of Ishizuka and Kunieda (**26a**). The carbamate was derivatized with di-*t*-butyloxycarbonyl anhydride (Boc_2O), followed by selective cleavage of the resulting dicarbamate with Cs_2CO_3 . This method has the distinct advantage over direct carbamate hydrolysis in that mild conditions are employed. Furthermore, the amino functionality of the resulting dipeptide analogue is Boc-protected, facilitating elaboration into a longer peptide derivative, the next step in the synthesis. However, when we attempted to cleave the cyclic carbamate **7** using this procedure (Scheme 3), only a 26% yield of the desired ring-opened product **14** was obtained (the major product being regenerated starting carbamate **7**). More distressingly, as shown in

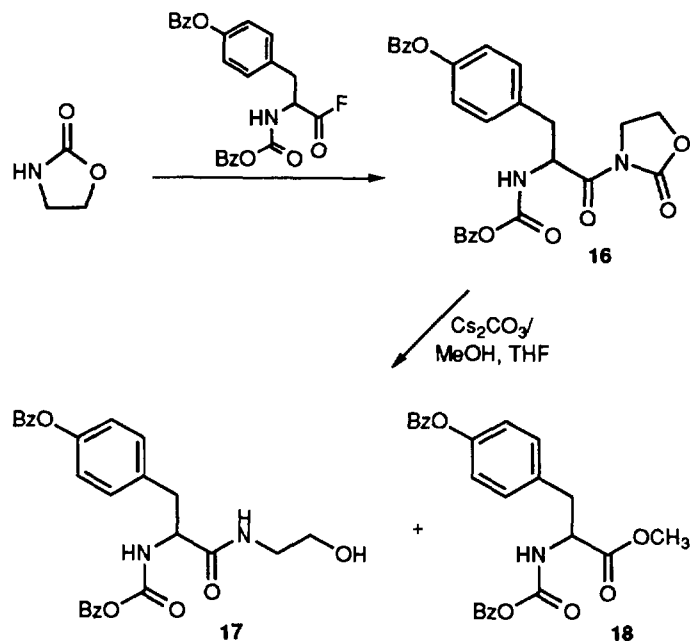
TABLE I
Endocyclic versus Exocyclic Cleavage with Cs_2CO_3 in a Series of Dicarbamates

|  | | |
|--|-------------------|------------------|
|  | 100% ^a | 0% ^a |
|  | 57% ^b | 43% ^b |
|  <p>13</p> | 37% | 63% |
|  <p>15</p> | 0% | 100% |

^a Ref. (20a).

^b D.P.W. and D.E.H., unpublished results.

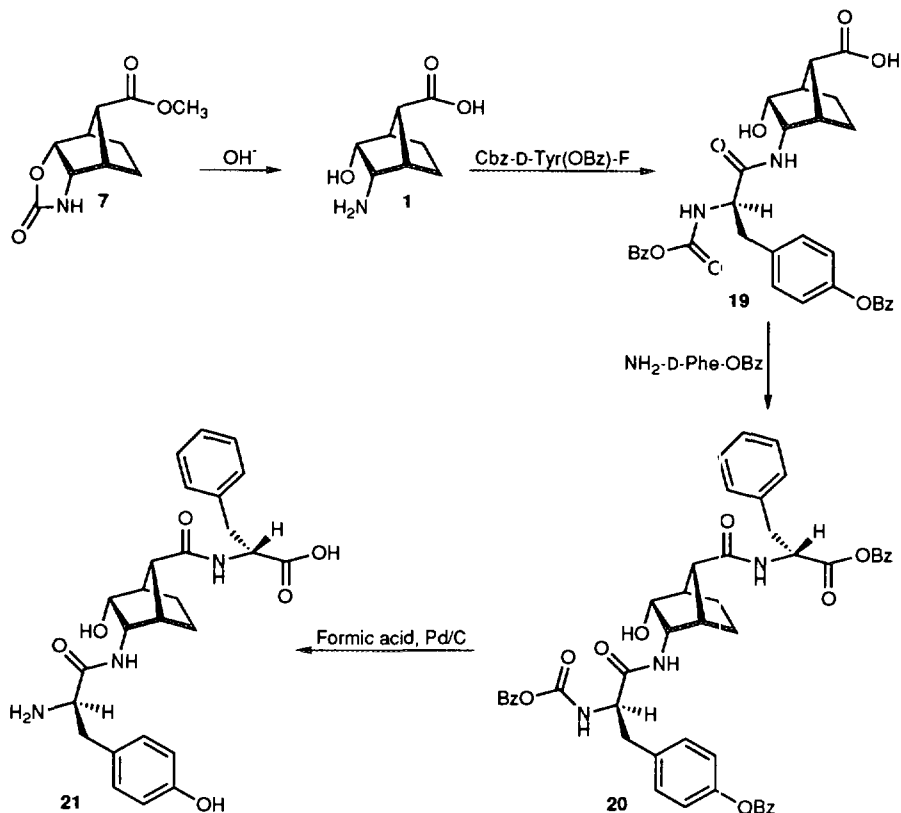
Table 1, with Boc-carbamate **15**, no ring-opened product was detected at all, the Cs_2CO_3 cleavage reaction yielding only starting carbamate. As is evident from the table, which also includes data obtained with a cyclobutyl and cyclopentyl derivative, Boc cleavage is favored over cyclic carbamate cleavage as steric crowding is in-



SCHEME 4

creased. Interestingly, blockage of one face of the cyclic carbamate apparently is sufficient to completely favor exocyclic carbamate cleavage. Regardless of the precise reasons, there is clearly a fine balance between endocyclic and exocyclic carbamate cleavage in sterically bulky derivatives, a balance that was not revealed in earlier studies of this reaction (26). Before turning to simple base-catalyzed hydrolysis of the carbamate, we explored a modification of Ishizuka and Kunieda's method: coupling of an amino acid residue to the carbamate, followed by selective cleavage. In a model study, Cbz-D-Tyr(Bzl)-F was coupled to oxazolidone to yield the product **16**, which was treated with Cs_2CO_3 in methanol (MeOH)/tetrahydrofuran (THF) (Scheme 4). However, the exocyclic cleavage again predominated, resulting in an approximately 1 to 5 ratio of the species **17** and **18**. (In a related system, Knapp *et al.* (27) found that benzoylcarbamates are also selectively hydrolyzed by LiOH at the benzoyl carbonyl group.)

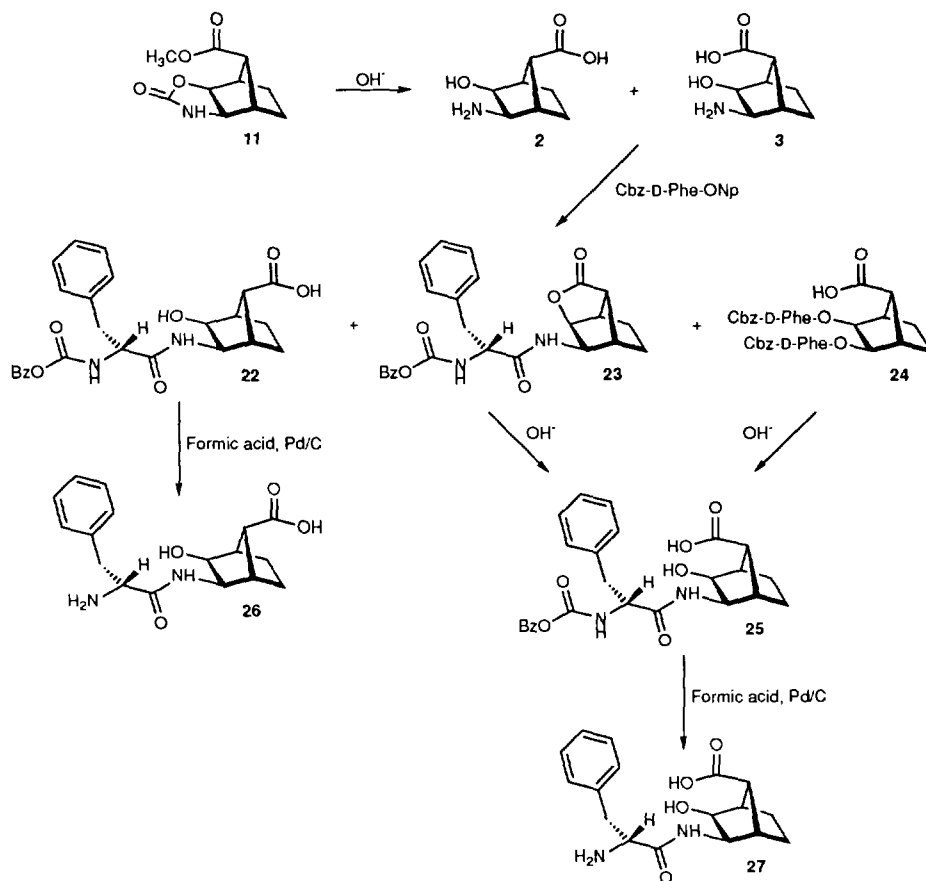
In view of the above results, we ultimately cleaved the carbamate esters in refluxing aqueous sodium hydroxide. As shown in Scheme 5, the carbamate ester **7** gave the expected dipeptide analogue **1**. As shown in Scheme 6, however, the carbamate ester **11** yielded both the anticipated *syn*-derivative **3** and the epimerized *anti*-derivative **2**. (Under identical conditions, no epimerization was observed during the hydrolysis of **7** above.) The observation of extensive epimerization in the hydrolysis of carbamate **11** illustrated the advantage of a mild method of carbamate



SCHEME 5

cleavage, such as the $\text{Cs}_2\text{CO}_3/\text{MeOH}$ procedure described above. In this instance, however, the epimerization provided direct access to compound **2**.

Amino acid couplings. The dipeptide analogue **1** was coupled to Cbz-D-Tyr(Bzl)-F with triethylamine (TEA) in dimethylformamide (DMF) to yield the protected peptide derivative **19** (Scheme 5) as a mixture of diastereomers. (D-Amino acids were chosen so that the peptide derivatives would be more immunogenic. Clearly, if antibodies with activity against the corresponding peptides are obtained, derivatives incorporating only L-amino acids will be prepared and the experiment repeated.) NaHCO_3 in THF also provided effective conditions for the formation of coupled product **19** in high yield. However, a two-phase solvent system of aqueous bicarbonate and CH_2Cl_2 (1:1) gave only very low yields of **19**, presumably because of the low solubility of **1** in CH_2Cl_2 . The coupling reaction was monitored by reverse-phase HPLC, which showed that no detectable coupling to the free hydroxyl group occurred in competition with the amino coupling. The tripeptide derivative **19** was then coupled using standard conditions (28) to D-phenylalanine benzyl ester to yield the derivative **20**, which upon deprotection yielded tetrapeptide analogue **21**.



SCHEME 6

In preparation for immunization, **21** was coupled to the heterobifunctional linker *N*-succinimidyl-6-maleimidocaproate, and the product conjugated to the carrier proteins bovine serum albumin and keyhole limpet hemocyanin.

The analogues **2** and **3**, which as discussed above were obtained as a mixture upon hydrolysis of the carbamate ester **11**, were elaborated only at the amino-terminus. Given the juxtaposition of the carboxylic acid functionality and the peptide bond mimicked in **3**, antibodies raised against this derivative may effect substrate-assisted exopeptide bond cleavage. That is, when bound to the antibody, the substrate may be held in a conformation that would allow its terminal carboxylate to participate in general base or nucleophilic catalysis. Such substrate-assisted catalysis cannot occur with antibodies elicited against the *anti*-derivative **2**, and these antibodies will provide an important control.

The mixture of **2** and **3** was directly coupled to Cbz-D-Phe-ONp in DMF. After workup and chromatography on silica gel, three products were obtained: the singly