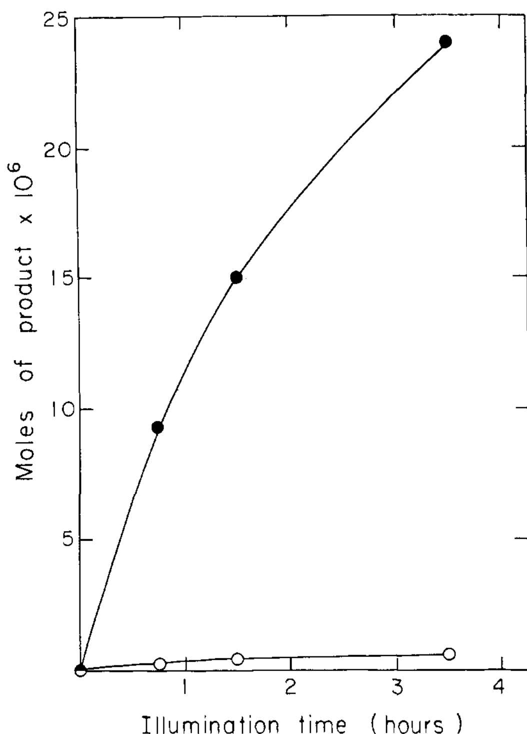

Figure 1. Rate of formate (●) and hydrogen (○) formation as a function of illumination time.

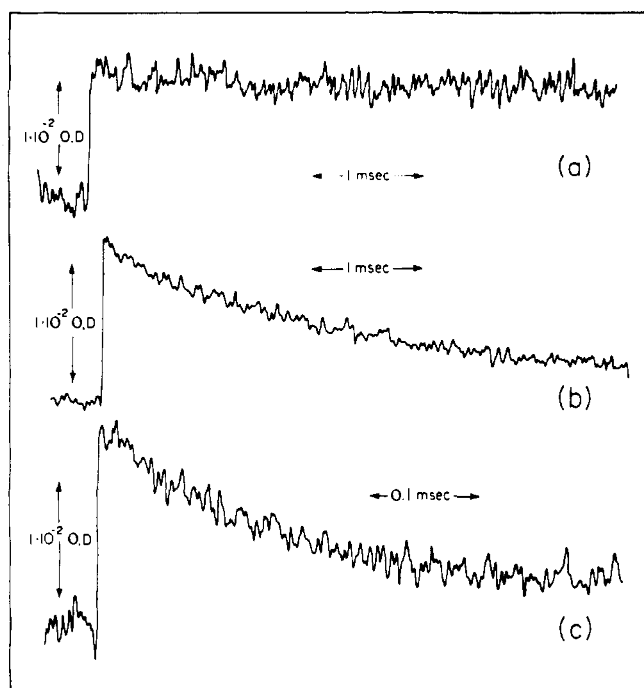

Figure 2. Transient decay of $MV^{\cdot+}$ followed at λ = 602 nm in systems composed of dRFl, 4×10^{-5} M; MV^{2+}, 1×10^{-3} M; and oxalic acid, 6.7 $\times 10^{-2}$ M. (a) Without CO_2/HCO_3^- or Pd-β-CD. (b) Without CO_2/HCO_3^- and with Pd-β-CD (30 mg·L^{-1}). (c) With CO_2/HCO_3^- (6.7×10^{-2} M) and with Pd-β-CD (30 mg·L^{-1}). All systems were adjusted to pH 7 and degassed by either CO_2 (b) and (c) or by Ar (a).

amount of Pd-β-CD catalyst included reveals that the catalyst performs ca. 10 turnovers.

It is evident that the Pd-β-CD colloid is a poor catalyst for H_2 evolution. Comparison of the quantum yields obtained under steady-state illumination of $MV^{\cdot+}$ production to those of HCO_2^- and H_2 formation suggests that the catalytic processes are the rate-limiting steps. Laser flash photolysis studies have confirmed that Pd-β-CD is a superior catalyst for CO_2/HCO_3^- reduction as compared to H_2 evolution (Figure 2). Flashing the system that includes dRFl, MV^{2+}, and oxalate results in the steady-state accumulation of $MV^{\cdot+}$ (Figure 2a) as a result of the photoreduction of MV^{2+}. Addition of Pd-β-CD in the absence of CO_2/HCO_3^- (Figure 2b) induced a slow decay of $MV^{\cdot+}$ due to H_2 evolution. Addition of CO_2/HCO_3^- (Figure 2c) to the system effects a rapid decay of $MV^{\cdot+}$, implying that the rate of CO_2/HCO_3^- reduction is substantially faster than H_2 evolution.

Formate, HCO_2^-, reduces MV^{2+} in the dark in the presence of Pd-β-CD (eq 1). This allows us to examine the catalytic activity of the Pd colloid in the presence of various additives, and particularly in the presence of sacrificial electron donors, by means of the reverse formate decomposition process. We find that common electron donors such as thiols and the photodecomposition products of EDTA (formaldehyde) inhibit the catalytic activity of Pd-β-CD toward formate decomposition. Accordingly, no photoreduction of CO_2/HCO_3^- is observed in the presence of these electron donors. Oxalate does not inhibit the catalytic activity of Pd-β-CD and explains the success to photoinduce the reduction of CO_2 in the present system.

In conclusion, we have developed an effective system for the photoreduction of CO_2/HCO_3^- to formate by visible light. It should be noted that the β-CD support for the Pd colloid is extremely important to its catalyst activity and Pd colloids prepared by the reduction with citrate or stabilized by polymers, i.e. poly vinyl alcohol, are inactive toward formate production. Previous studies[19] have indicated that hydroxyl-containing supports, i.e., alumina, participate cooperatively in the activation of CO_2 by Pd metal. Similarly, cyclodextrins have been claimed[20] to associate CO_2, and derivatized cyclodextrins catalyze the hydration of CO_2.[21] The possible cooperative activation of CO_2/HCO_3^- by β-CD and Pd are now being investigated.

Acknowledgment. This research is supported by the Belfer Center for Energy Research, Israel.

Registry No. 1, 19342-73-5; CO_2, 124-38-9; HCO_3, 71-52-3; HCOOH, 64-18-6.

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Incorporation of D-Amino Acids into Peptides via Enzymatic Condensation in Organic Solvents

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A number of biologically active peptides, including important antibiotics, synthetic vaccines, and enkephalins and other hormones, contain D-amino acid residues.[1] Although enzymes, namely proteases, are becoming increasingly popular as catalysts of peptide bond formation,[2] this synthetic methodology (as well

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Table I. Synthesis of Peptides Containing D-Amino Acids Catalyzed by Subtilisin in Anhydrous *tert*-Amyl Alcohol[a]

substrates[b] (amt, mmol)			
amino acid ester	nucleophile	product[c]	isold yield of product, %
N-Ac-D-Phe-OEtCl (3.1)	L-Phe-NH_2 (3.1)	*N*-Ac-D-Phe-L-NH_2[d]	67
N-F-D-Ala-OEtCl (5.1)	L-Phe-NH_2 (14.6)	*N*-F-D-Ala-L-Phe-NH_2[e]	82
N-Ac-D-Asn-OEtCl (5.0)	L-Le-NH_2 (7.5)	*N*-Ac-D-Asn-L-Leu-NH_2[f]	71
N-Ac-D-Trp-OEtCl (8.4)	L-Phe-NH_2 (8.4)	*N*-Ac-D-Trp-L-Phe-NH_2[g]	47
N-F-D-Ala-OEtCl (6.0)	D-Ala-NH_2 (6.0)	*N*-F-D-Ala-D-Ala-NH_2[h]	65
N-F-D-Ala-OEtCl (16.0)	L-Phe-L-Leu-NH_2 (16.0)	*N*-F-D-Ala-L-Phe-L-Leu-NH_2[i]	61
N-CBZ-L-Tyr-OEtCl (2.5)	D-Ala-NH-$(CH_2)_3$-Ph (7.8)	*N*-CBZ-L-Tyr-D-Ala-NH-$(CH_2)_3$-Ph[j]	54

[a] The amounts of the substrates given in Table I were dissolved in anhydrous *tert*-amyl alcohol (31, 51, 30, 42, 15, 100, and 25 mL, respectively, in the order of entries from top to bottom), followed by addition of 3.3 mg/mL of subtilisin.[6] The suspension (the enzyme is insoluble in *tert*-amyl alcohol) was stirred at 45 °C for a certain period of time (see below). The solvent was then evaporated under vacuum, the residue was thoroughly washed with water, and the product was twice recrystallized from warm methanol. In no case was any peptide formation detected (by HPLC) in the absence of subtilisin. [b] Nonstandard abbreviations: Ac = acetyl, F = formyl, OEtCl = 2-chloroethyl ester. [c] All isolated products were crystalline compounds pure by TLC and HPLC. The ^{1}H NMR spectra (250 MHz) for all products were consistent with their proposed structures. In the case of the product listed in the first entry of Table I, we established that no racemization occurred during enzymatic peptide bond formation: the *N*-Ac-D-Phe-L-Phe-NH_2 formed was compared with the previously enzymatically prepared by us (see ref 4a) *N*-Ac-L-Phe-L-Phe-NH_2. The two diastereomers had well resolved ^{1}H NMR spectra and their HPLC retention times differed by 0.5 min under the conditions used (μBondapak C_{18} column, isocratic regime, 30% acetonitrile–70% aqueous buffer (10 mM phosphate–triethylamine, pH 2.6) as the mobile phase, 1 mL/min flow rate). No all-L peptide impurity was detected in the D–L dipeptide by either method (the sensitivities were better than 5% and 3%, respectively). [d] Mp 230–232 °C, $[\alpha]_D^{25}$ −15.2° (*c* 0.4, MeOH), 0.73 g of the product after a 3-day reaction. Anal. Calcd for $C_{20}H_{23}N_3O_3$: C, 67.99; H, 6.52; N, 11.90. Found: C, 67.69; H, 6.54; N, 11.73. [e] Mp 198–200 °C, $[\alpha]_D^{25}$ +3.9° (*c* 0.6, DMF), 1.1 g of the product after a 3-day reaction. Anal. Calcd for $C_{13}H_{17}O_3N_3$: C, 59.31; H, 6.46; N, 15.97. Found: C, 59.17; H, 6.42; N, 16.10. [f] Mp 216–218 °C, $[\alpha]_D^{25}$ −20.0° (*c* 0.4, DMF), 1.0 g of the product after a 4-day reaction. Anal. Calcd for $C_{12}H_{22}N_4O_4$: C, 50.35; H, 7.69; N, 19.58. Found: C, 50.61; H, 7.55; N, 19.34. [g] Mp 265–267 °C, $[\alpha]_D^{25}$ −32.9° (*c* 0.16, MeOH), 1.5 g of the product after a 4-day reaction. Anal. Calcd for $C_{22}H_{24}N_4O_3$: C, 67.35; H, 6.12; N, 14.29. Found: C, 67.18; H, 6.20; N, 13.93. [h] Mp 184–186 °C, $[\alpha]_D^{25}$ +92.5° (*c* 0.2, MeOH), 0.72 g of the product after a 3-day reaction. Anal. Calcd for $C_7H_{11}N_3O_3 \cdot {}^1/_2H_2O$: C, 43.32; H, 6.19; N, 21.65. Found: C, 43.57; H, 6.15; N, 21.57. [i] Mp 256–258 °C, $[\alpha]_D^{25}$ −29.8° (*c* 0.6, DMF), 3.65 g of the product after a 4-day reaction. Amino acid analyses obtained for this tripeptide after hydrolysis with 6 N HCl at 110 °C for 24 h were as follows: Ala (1) 1.00; Phe (1) 1.00; Leu (1) 1.01. The dipeptide L-Phe-L-Leu-NH_2 used as a nucleophile was prepared enzymatically as described in the text and had the following characteristics:[9] mp 113–115 °C, $[\alpha]_D^{25}$ −17.5° (*c* 0.2, MeOH). Anal. Calcd for $C_{15}H_{23}N_3O_2$: C, 64.98; H, 8.30; N, 15.16. Found: C, 64.79; H, 8.35; N, 14.97. [j] Mp 218–220 °C, $[\alpha]_D^{25}$ +38.0° (*c* 0.2, MeOH), 0.68 g of the product after a 5-day reaction. Anal. Calcd for $C_{29}H_{33}N_3O_5$: C, 68.18; H, 6.56; N, 8.35. Found: C, 67.98; H, 6.74; N, 8.14. The product was deprotected by a standard method to afford 0.45 g of pure L-Tyr-D-Ala-NH-$(CH_2)_3$-Ph which is known[10] to possess an enkephalin activity.

as recombinant DNA technology) is not generally applicable to peptides involving D-amino acids because of the L-specificity of proteolytic enzymes. The only exception has been the use of D-amino acid derivatives as nonspecific nucleophiles competing with water for the acyl enzyme formed in the reaction between a protease and a protected L-amino acid ester.[3] This approach, however, is inherently limited to the enzymatic incorporation of a single D-amino acid residue into a peptide's C-terminal position.[3] It remains to be seen whether this restriction may be overcome even by the recently elaborated use of nonproteases, lipases, as catalysts of peptide synthesis.[4]

Over the last few years it has transpired that not only can enzymes function as catalysts in organic solvents instead of water, but when placed in this unnatural medium they exhibit novel catalytic properties.[5] In the present study, we found that upon a transition from aqueous solutions to anhydrous organic solvents, the discriminating stereoselectivity of the protease subtilisin drastically relaxed, and consequently this enzyme was used to prepare numerous peptides containing D-amino acids in various positions.

We began by establishing that subtilisin[6] was catalytically competent in a variety of anhydrous organic solvents;[7] the initial rates of the enzymatic reaction between *N*-acetyl-L-phenylalanine chloroethyl ester and L-leucine amide in *tert*-amyl alcohol, tetrahydrofuran, acetone, dichloromethane, pyridine, dimethylformamide (DMF), toluene, acetonitrile, ethyl acetate, and dioxane were found to be 123, 104, 113, 83, 84, 0.9, 1.4, 47, 72, and 14 nmol/min·mg enzyme, respectively (50 mM substrates, 45 °C, shaking at 250 rpm, formation of the dipeptide product followed by HPLC). (No appreciable reaction was observed under the same conditions with subtilisin preinactivated[8] with phenylmethanesulfonyl fluoride.) The enzymatic peptide bond synthesis was scaled up by using the optimal solvent (*tert*-amyl alcohol) and the easily removable CBZ group (instead of acetyl). As a result, 7.3 g (88% overall isolated yield) of L-Phe-L-Leu-NH_2[9] was obtained after a 4-day reaction following deprotection. It is worth noting that no dipeptide synthesis (only ester hydrolysis) was observed in water (10 or 40% DMF, pH 7 or 10) under identical conditions.

In aqueous solutions subtilisin is highly stereoselective: e.g., the initial rate of enzymatic hydrolysis of the L isomer (ν_L) of *N*-Ac-Phe-OEtCl was found to exceed that for the D-isomer (ν_D) by 773-fold; for the alanine ester ν_L/ν_D = 204 (50 mM esters, 40% DMF, pH 7, 45 °C, measured potentiometrically). In contrast, the ν_L/ν_D ratios for the aforementioned peptide synthesis reaction in *tert*-amyl alcohol for the *N*-acetyl-Phe and Ala esters were as low as 7.6 and 2.8, respectively. Similar data were obtained in acetone. The comparable reactivities of L- and D-isomers in organic solvents led to the first enzymatic synthesis of peptides containing D-amino acids in the *N*-terminal position.

Table I depicts the results of subtilisin-catalyzed preparative synthesis of seven different peptides in anhydrous *tert*-amyl alcohol. One can see that (i) various D-amino acids (Ala, Phe, Trp, and Asn) were readily enzymatically incorporated in the first position of peptides; (ii) the enzyme is quite tolerant to the nature of

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(11) This work was financially supported by W. R. Grace & Co.

protective groups (CBZ, formyl, and acetyl), including the easily removable ones; and (iii) various amino acid derivatives including both L- and D-isomers and dipeptides were utilized as nucleophiles.

In summary, a radically altered stereospecificity of subtilisin in organic solvents affords facile enzymatic preparation, impossible in water, of diverse peptides containing D-amino acids, thus providing a new synthetic route to bioactive peptides.

Thermal Generation and Dimerization of [4]Metacyclophane

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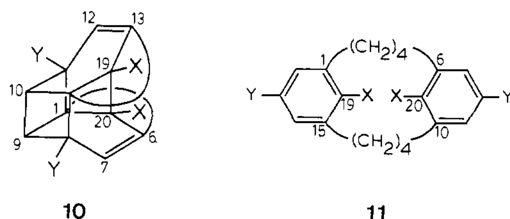
The field of cyclophanes with very short bridges continues to bring forth intriguing results.[1] While [5]metacyclophane (1977)[2] and [5]paracyclophane (1985)[3] have already been synthesized, attempts to bridge a benzene ring with four atoms have not yet yielded an isolable compound. [4]Paracyclophane, predicted by Schaefer et al. "on statistical grounds" to be synthesized in 1992,[4] has recently been generated photochemically at low temperatures, intercepted with alcohols,[5,6] and identified by its UV spectrum.[6] [4]Metacyclophane (**2a**) is expected to be less strained and more stable than its para isomer and thus appears to be overdue in this series.

Our previous attempts[7] to obtain **2a** by irradiation of its Dewar isomer **1a**[8] were unsuccessful because **1a** furnished the prismane isomer in a quantitative escape reaction. In the course of those studies, we observed on GCMS small quantities of compounds with double mass. An investigation of the dimer formation has now furnished good evidence for the involvement of **2a** and has revealed some fascinating reactions and products.

Depending on the thermolysis conditions, **1** yielded different product mixtures (see Scheme I and Table I). Compounds **3** and **12**[9] were known. The structures of **10** and **11** could be assigned



from their spectral data and from those of deuteriated derivatives.[10]

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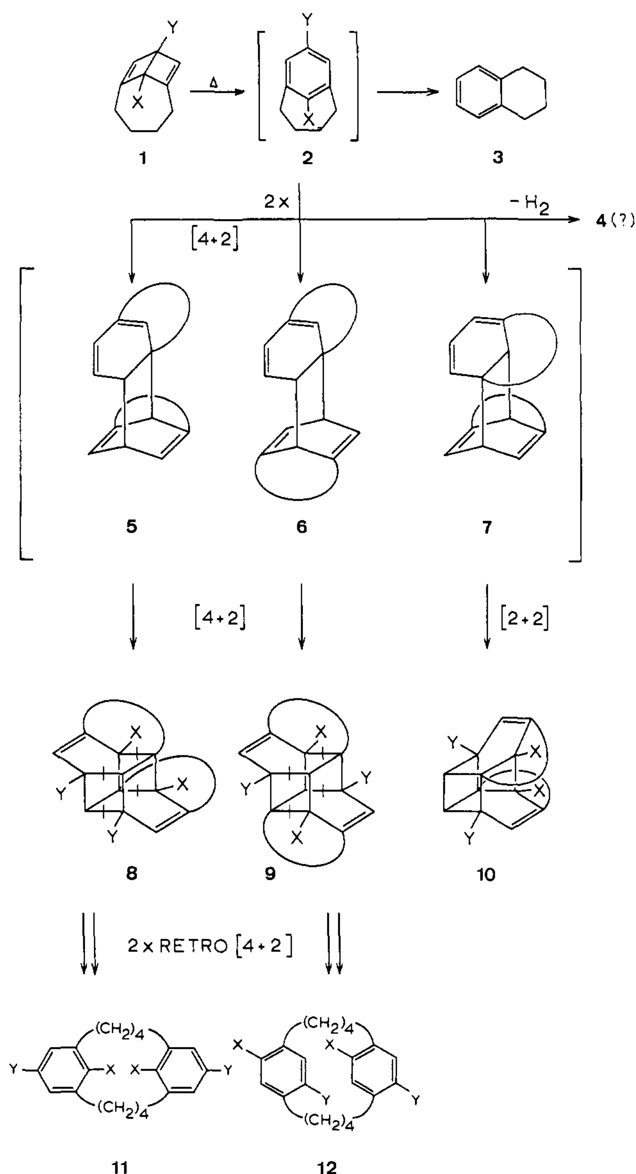
Scheme I[a]



[a] **a**; X = Y = H, **b**; X = D, Y = H, **c**; X = H, Y = D.

The dehydrogenated dimer **4** was not further characterized. Unfortunately, **8**, **9**, and probably three other dimers formed in comparable amounts turned out to be so similar in their physical properties that we have not yet been able to separate them by HPLC or by preparative GC; the latter technique suffers from the additional disadvantage that some decomposition occurred at the high injector temperatures required due to the low volatilities of the products. Therefore, their structural assignment is tentative; it is based on GCMS and a few typical ^{1}H NMR signals,[10] and on their thermal behavior.

When **1** was injected into the gas chromatograph (injector temperature 300 °C, Table I, entry 1), **10a** was the main component of the product mixture. Heating **1a** at 200 °C in a KOH-conditioned[11] sealed ampoule gave, besides much polymer, the same substances, but with drastically changed product ratios, and **12a** as the main component (77%; entry 2). Mechanistically revealing were the ampoule experiments at lower temperatures (entries 3 and 4); here, according to GCMS and NMR analysis, a mixture of at least five dimers, including **8a** and **9a**, was obtained.

(10) Spectral data and assignments are available as Supplementary Material.

(11) Reactions in unconditioned ampoules gave variable results; in most cases, large amounts of **3** (up to 60%) were obtained. In a run with 2 mol % *p*-toluenesulfonic acid, the yield of **3** rose to 90%.

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