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PEPTIDE BOND FORMATION VIA CATALYTIC ANTIBODIES: SYNTHESIS OF A NOVEL PHOSPHONATE DIESTER HAPTEN

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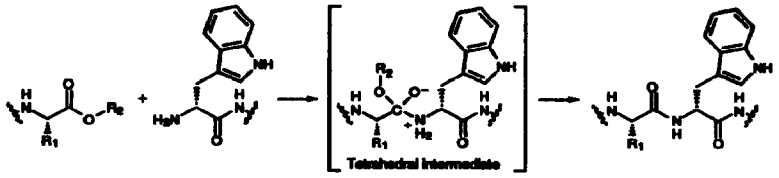
Abstract: Phosphonate diester **2**, a hapten recently employed to induce the first monoclonal catalytic antibodies capable of catalyzing peptide bond formation, was synthesized via elaboration of scalemic α-amino phosphonate **4**.

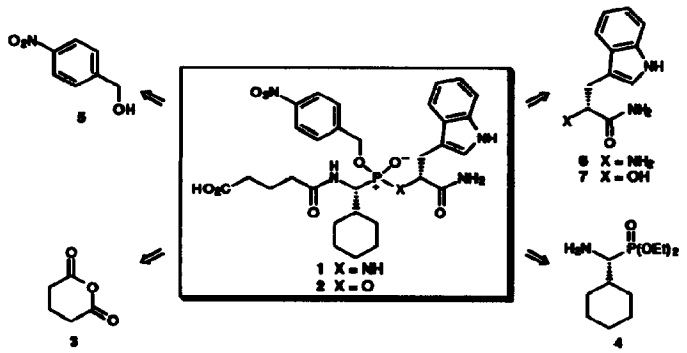
Although peptide fragment condensation (Figure 1) remains a principal chemical strategy for large protein synthesis,[1] the approach suffers from significant drawbacks, including low yields and slow coupling rates for large peptide fragments, and the requirement for multiple hydrophobic protecting groups and the resultant insolubility of the reactants. To circumvent these problems we recently established a collaborative venture aimed at exploiting catalytic antibodies for

Figure 1. Classical Condensation of Peptides.



peptide fragment coupling. Although many successful applications of catalytic antibodies to organic transformations have been described,[2,3] the successful formation of a peptide bond via antibody catalysis had not been documented.[4] To this end, we designed hapten **1**; our design represents a significant departure from the tradition of exact transition state mimicry.[5] Whereas the synthesis of **1** remains elusive *(vide infra)*, the analogous phosphonate diester **2** was prepared and utilized to induce two monoclonal antibodies which effectively catalyze peptide bond formation.[5] In this Letter we report the synthesis of hapten **2**.

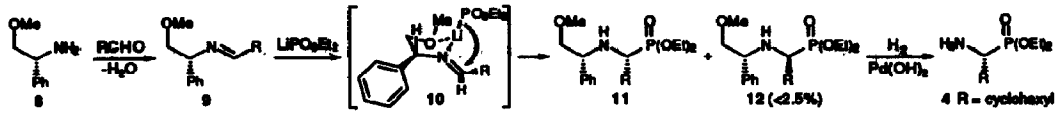
Scheme 1



Our synthetic approach to **1** and **2** involves elaboration of 1-amino-1-cyclohexylmethylphosphonic acid diethyl ester (**4**), as outlined in Scheme 1. Key steps include: (a) preparation of scalemic α-aminophosphonate **4**; (b) generation of the *p*-nitrobenzyl ester; (c) installation of a suitable phosphonamide group (for **1**) or a second phosphonate ester linkage (for **2**); and (d) N-acylation with glutaric anhydride.

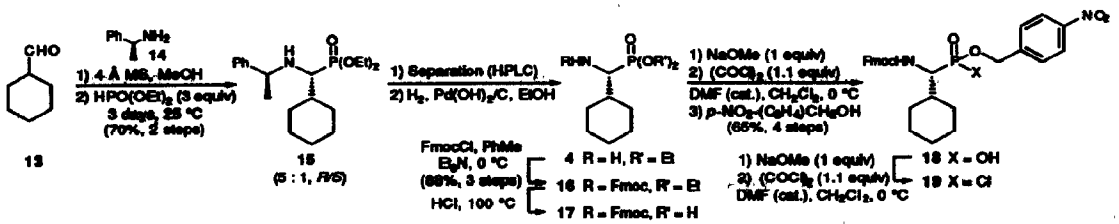
As our point of departure *(S)*-α-phenethylamine was condensed with cyclohexanecarboxaldehyde to furnish the corresponding imine. Addition of diethyl phosphite[6] then afforded a 5:1 mixture of the diastereomeric amines **15**;[7] unfortunately, isomer separation by preparative HPLC [silica gel (21 mm column); EtOAc/hexanes (3:2), 16 mL/min] proved to be exceedingly laborious. The need for a more expedient preparation prompted us to explore the asymmetric synthesis of α-amino phosphonates via addition of lithium dialkyl phosphites to scalemic chelating imines. This approach (Scheme 2) furnished the target compounds with excellent diastereoselectivity (>95% de), as described in detail elsewhere.[8]

Scheme 2



In the present study, hydrogenolysis of the major isomer of **15** removed the chiral auxiliary, furnishing the primary amine **4**.[7a,9] X-ray analysis of the derived hydrochloride salt confirmed the desired *R* configuration.[10] The amine group was next protected as the fluorenylmethoxycarbonyl (Fmoc) derivative. We expected the carbamate protecting group to minimize racemization during the subsequent coupling reaction, a technique amply documented in peptide synthesis. Phosphonic acid **17**[7] was obtained via hydrolysis of **16**[7] in hot concentrated hydrochloric acid. Treatment of the monosodium salt of **17** with oxalyl chloride and a catalytic amount of DMF[11] followed by reaction with *p*-nitrobenzyl alcohol furnished monoester **18**[7] after HPLC purification. The latter intermediate in turn furnished phosphonochloridate **19**.

Scheme 3

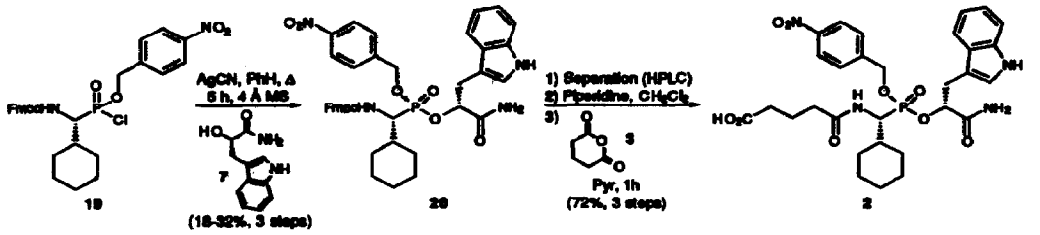


Attempts to couple **19** with several tryptophan derivatives, as well as with simpler primary amines, were uniformly unsuccessful. We believe that amines react with the phosphonochloridate in a way, which precludes coupling in the desired sense.[12] Studies directed towards understanding the mechanistic basis for this are in progress and will be reported in due course.

In contrast, a variety of alcohols (MeOH, EtOH, i-PrOH, t-BuOH, $PhCH_2OH$ and tryptophol) reacted smoothly with **19** at room temperature. Coupling with the requisite β-indolyllactamide **7**[7,13] required more stringent conditions, probably a consequence of the electron-withdrawing amide functionality; we therefore turned to the silver cyanide protocol introduced by Takimoto et al.[14] in 1976. Indeed, use of a stoichiometric amount of silver cyanide in benzene, at reflux, led to formation of diester **20**[7a] (Scheme 4). Although we were unable to improve upon the modest yield of the coupling, we believe that

this represents the first example of silver-promoted phosphonate ester formation.

Scheme 4



Diester **20** was generated as a 3.6:1 mixture of diastereomers which were separated by RP-HPLC. The major isomer was employed in all subsequent studies; as yet, we do not know the relative stereochemistry at phosphorus. Removal of the Fmoc group with piperidine gave the primary amine, which upon acylation with glutaric anhydride furnished hapten **2**.[7a]

In summary, we have developed an effective synthesis of hapten **2**, which upon conjugation to a carrier protein and immunization produced monoclonal antibodies capable of catalyzing peptide bond formation.[5] This work represents the first documented antibody-catalyzed formation of a peptide bond, and as such suggests possible routes towards creating a small number of antibody catalysts for polypeptide synthesis.

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7. (a) The structure assigned to each new compound was in accord with its infrared, 500-MHz ^{1}H NMR and 125-MHz ^{13}C NMR spectra, as well as appropriate parent ion identification by high resolution mass spectrometry. (b) In addition, an analytical sample of this compound, obtained by recrystallization or liquid chromatography, gave satisfactory combustion analysis within 0.4%.
8. (a) Yager, K. M.; Taylor, C. M.; Smith, A. B., III *J. Am. Chem. Soc.* (submitted for publication).

9. (-)-4: colorless oil; $[\alpha]_D^{25}$ -52.2° (*c* 1.2, acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13-2.04 (m, 11H), 1.34 (m, 6H), 2.83 (dd, J_{HH} = 4.3 Hz, J_{HP} = 14.3 Hz, 1H), 4.14 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.9, 16.2, 16.3, 25.8, 25.9, 26.2, 27.4, 27.5, 30.4, 30.5, 38.9, 53.6 (d, J_{CP} = 145 Hz), 60.1, 61.5, 61.6.

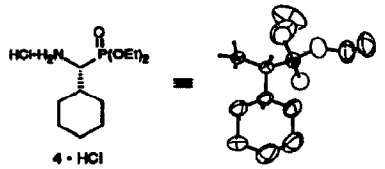
(-)-18: amorphous white solid, mp 186-187 °C (decomp.); $[\alpha]_D^{25}$ -7.0° (*c* 0.73, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.05-1.21 (m, 5H), 1.55-1.90 (m, 6H), 3.79 (m, 1H), 4.10 (m, 2H), 4.23 (dd, J_{HP} = 8.6 Hz, J_{HH} = 5.9 Hz, 1H), 5.05 (d, J_{HP} = 6.1 Hz, 2H), 7.26 (t, *J* = 7.5 Hz, 2H), 7.38 (td, *J* = 7.4, 3.0 Hz, 2H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.71 (d, *J* = 7.5 Hz, 2H), 7.86 (d, *J* = 7.5 Hz, 2H), 8.04 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.57, 25.66, 25.73, 28.3, 30.3, 30.4, 38.0, 46.6, 52.9 (d, J_{CP} = 154 Hz), 64.8, 65.8, 120.0, 123.2, 123.3, 125.3, 126.90, 126.93, 127.50, 127.54, 127.6, 140.6, 143.7, 156.4; UV (EtOH) [λ (nm) (ε)] 206 (81,800), 265 (42,500), 299 (14,500).

(+)-20: (minor isomer): R_T 23.5 min; $[\alpha]_D^{25}$ = +15.3° (*c* = 0.47, EtOH); ^{1}H NMR (500 MHz, $CHCl_3$) δ 0.65-1.85 (m, 11H), 3.33 (m, 2H), 3.95 (ddd, *J* = 4.5, 10.7, 18.5 Hz, 1H), 4.12 (t, *J* = 6.0 Hz, 1H), 4.35 (dd, *J* = 6.0, 10.8 Hz, 1H), 4.60 (dd, *J* = 6.0, 10.8 Hz, 1H), 4.60 (d, *J* = 10.7 Hz, 1H), 4.97-5.06 (m, 3H), 5.54 (br s, 1H), 6.12 (br s, 1H), 7.00 (d, *J* = 1.8 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 7.15 (t, *J* = 7.5 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.53 (dd, *J* = 7.6, 10.8 Hz, 2H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.78 (dd, *J* = 2.0, 7.5 Hz, 2H), 7.96 (br s, 1H), 8.06 (d, *J* = 8.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.6, 25.7, 25.8, 27.8, 29.2, 29.7, 30.3, 30.4, 38.0, 38.1, 47.2, 53.3 (d, J_{CP} = 154 Hz), 66.6, 66.8 (d, J_{CP} = 7 Hz), 109.3, 111.2, 118.7, 119.8, 120.0, 120.1, 122.3, 123.7, 124.6, 124.8, 127.1, 127.2, 127.5, 127.8, 127.9, 128.1, 135.9, 141.3, 141.4, 142.7, 143.5, 143.6, 147.8, 156.1, 171.8; UV (EtOH) [λ (ε)] 212 (362,000), 266 (213,000), 289 (94,000), 300 (56,000).

(+)-20: (major isomer): R_T 24.6 min; $[\alpha]_D^{25}$ = +24.9° (*c* 0.44, EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.46-1.64 (m, 11H), 3.18 (dd, *J* = 9.2, 15.0 Hz, 1H), 3.39 (d, *J* = 15.0 Hz, 1H), 3.81 (ddd, *J* = 6.2, 11.0, 17.5 Hz, 1H), 4.13 (d, *J* = 10.5 Hz, 1H), 4.18 (t, *J* = 5.1 Hz, 1H), 4.44 (dd, *J* = 5.1, 10.9 Hz, 1H), 4.67 (dd, *J* = 6.9, 12.9 Hz, 1H), 4.71 (dd, *J* = 5.3, 11.0 Hz, 1H), 4.82 (dd, *J* = 7.1, 12.8 Hz, 1H), 4.93 (m, 1H), 5.50 (br s, 1H), 6.71 (br s, 1H), 6.76 (d, *J* = 2.2 Hz, 1H), 7.03 (ddd, *J* = 2.5, 5.2, 7.9 Hz, 1H), 7.30-7.55 (m, 10H), 7.58 (dd, *J* = 7.4, 17.7 Hz, 2H), 7.84 (dd, *J* = 7.5, 19.0 Hz, 2H), 8.06 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.7, 25.8, 28.0, 28.1, 29.0, 30.3, 30.4, 38.0, 47.4, 52.8, (d, J_{CP} = 151 Hz), 66.0, 66.5, 110.0, 111.3, 118.6, 119.9, 120.1, 120.2, 122.4, 122.8, 123.7, 124.6, 124.9, 127.3, 127.7, 127.9, 128.1, 135.7, 141.3, 141.5, 142.5, 142.6, 143.5, 143.9, 147.7, 156.2, 172.0.

(+)-2: yellow oil; $[\alpha]_D^{25}$ = +27.5° (*c* 0.38, EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.00-1.20 (m, 5H), 1.55-1.75 (m, 6H), 1.91 (t, *J* = 7 Hz, 2H), 2.25-2.50 (m, 4H), 3.22 (dd, *J* = 8.6, 15.5 Hz, 1H), 3.37 (dd, *J* = 3.5, 15.5 Hz, 1H), 4.14 (dd, *J* = 6.7, 13.0 Hz, 1H), 4.53 (dd, *J* = 7.5, 13.0 Hz, 1H), 4.59 (ddd, *J* = 4.4, 9.7, 19.3 Hz, 1H), 5.11 (td, *J* = 3.5, 8.8 Hz, 1H), 6.64 (s, br, 1H), 6.99 (d, *J* = 8.7 Hz, 2H), 7.05-7.20 (m, 4H), 7.25 (br s, 1H), 5.29 (d, *J* = 8.0 Hz, 1 H), 7.42 (d, *J* = 10.2 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 8.04 (d, *J* = 8.7 Hz, 2H), 8.55 (s, br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.5, 25.6, 25.9, 28.0, 29.7, 30.3, 33.3, 38.7, 50.2 (d, J_{CP} = 154 Hz), 66.0, 109.5, 111.4, 118.6, 119.8, 122.3, 123.6, 127.5, 135.9, 142.2, 147.6, 173.4, 174.0, 176.6; UV (EtOH) [λ (nm) (ε)] 220.4 (26,000), 269.6 (13,000).

10. Unpublished results of Dr. Patrick J. Carroll, University of Pennsylvania.



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