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A General Method for the Synthesis of *O*-Alkyl *N,O'*-Arylphosphoramidates and its Application to the Synthesis of a Transition State Analogue for Carbamate Hydrolysis.

Scott D. Taylor,*[a] Mei-Jin Chen,[a] A. Nicole Dinaut,[a] and Robert A. Batey[b]

[a]Department of Chemistry, University of Toronto, Mississauga Campus, 3359 Mississauga Rd. N., Mississauga, Ontario, Canada, L5L 1C6

[b]Department of Chemistry, University of Toronto, St. George Campus, 80 St. George St., Toronto, Ontario, Canada, M5S 3H6

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Abstract: *O*-alkyl *N,O'*-arylphosphoramidates were synthesized by reacting phenol and aniline derivatives with alkyldichlorophosphites to form phosphoramidites followed by oxidation with *m*-CPBA. Selective cleavage of the alkyl group under mild, neutral conditions afforded the corresponding *N,O*-arylphosphoramidic acids. This methodology was used to synthesize a *N,O*-arylphosphoramidate transition state analogue for carbamate hydrolysis.

INTRODUCTION

One of the problems associated with most cancer chemotherapeutic reagents is their limited ability to differentiate between cancer cells and healthy cells. To circumvent this problem, researchers have focussed on developing methods for delivering anticancer agents specifically to cancer cells. One such approach currently being investigated is known as antibody-directed abzyme prodrug therapy (ADAPT).[1,2] This procedure requires a protein conjugate consisting of an antibody that is specific for tumor-associated antigens and a catalytic antibody capable of prodrug activation.[3] When administered, the tumor-specific component of the conjugate recognizes tumor associated antigens and binds to the tumor cells. Once unbound conjugate is cleared, a non-toxic prodrug is administered. The prodrug is activated by the catalytic antibody portion of the conjugate to form the bioactive cytotoxic reagent. This results in a high local concentration of the drug in the vicinity of the cancer cells while minimizing its presence at healthy cells.

The first step in the development of an ADAPT system is the generation of antibodies capable of catalyzing the conversion of prodrugs into cytotoxic agents. It is now well established that antibodies raised to transition state analogues (TSA's) can catalyze chemical reactions[4,5] and several reports have recently appeared describing antibodies capable of converting prodrugs into cytotoxic agents using this methodology.[2,6,7] However, the drug or structural analogues of the drug were incorporated into the TSA's thus limiting them to the activation of a single drug.[2,6,7] We wished to develop a more flexible ADAPT system *in which a variety of prodrugs could be activated by a single catalytic antibody*. Consequently, bis-carbamate

staylor@credit.erin.utoronto.ca

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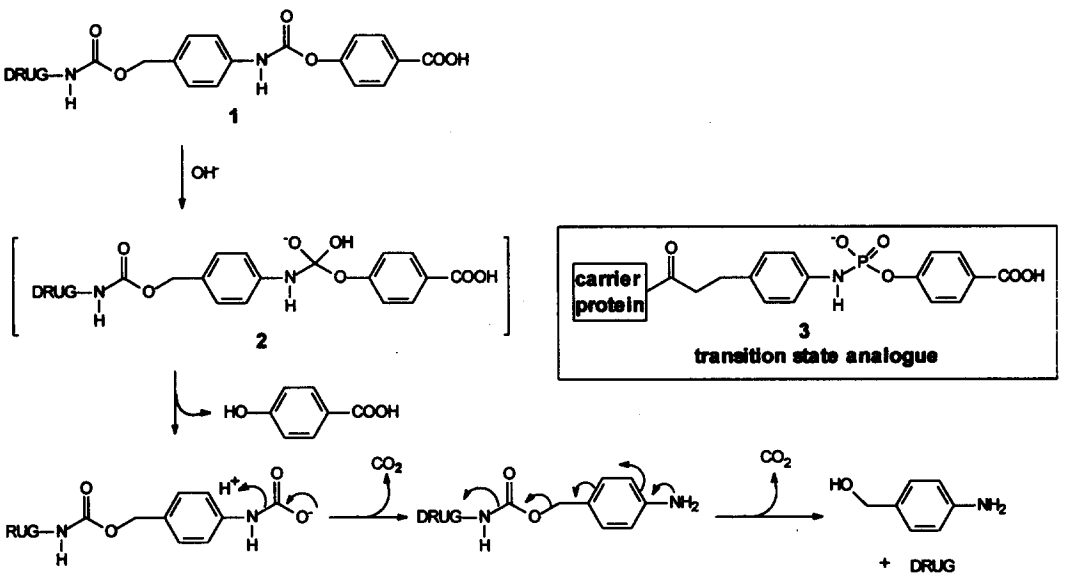
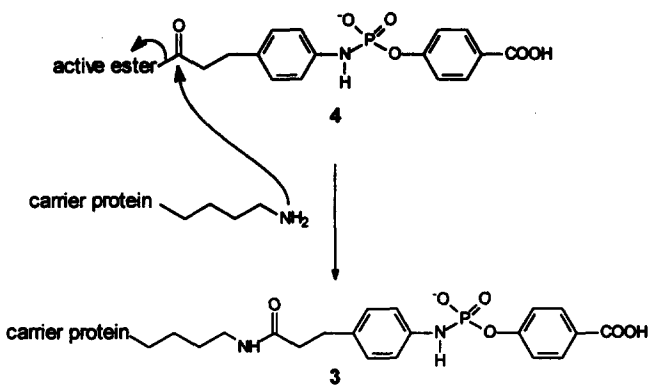


Scheme 1

prodrug **1** was designed as a model system.[8] Upon hydrolysis of the *N,O*-aryl carbamate component, the hydrolysis product undergoes further breakdown by an electron relay system[10a] to release an active drug (Scheme 1).[10b] Antibodies raised to phosphonamidate TSA's have been shown to catalyze the hydrolysis of carbamates[2,11,12] by a $B_{ac}2$ mechanism[11] and one of these antibodies has recently been employed in ADAPT.[2,11] However, we reasoned that superior carbamase abzymes should be obtained with a phosphoramidate hapten, such as **3** (Scheme 1), as opposed to a phosphonamidate hapten, since phosphoramidates should be better mimics of the rate determining transition state (**2** in Scheme 1) of the $B_{ac}2$ mechanism than phosphonamidates. The drug component of the prodrug is not incorporated into **3**. Instead, a carrier protein (necessary for antibody production and screening) is attached to a position on the TSA that is equivalent to the point of attachment of the drug to the carbamate substrate. Since antibodies are insensitive to changes in the region where the TSA is attached to the carrier protein[13] antibodies raised to **3** should only bind the "non-drug" portion of the prodrug substrate. By not incorporating the drug into the TSA, the drug should be free to enter the cell rather than bind to the abzyme and a variety of prodrugs could be activated by a single catalytic antibody.

In order to construct **3** it was necessary to devise a strategy for synthesizing the *N,O*-arylphosphoramidate TSA **4**, which has an activated ester moiety for the attachment of the carrier protein (Scheme 2). A review of the literature revealed that there is only a handful of reports describing the synthesis of *N,O*-arylphosphoramidic acids. The vast majority of the methods reported are based on phosphate chemistry utilizing *N*-aryldichlorophosphoramidates or *O*-aryldichlorophosphates as key reagents.[14,15] However, the synthesis of *N*-arylphosphoramidic dichloridates or *O*-arylphosphoric dichloridates can be

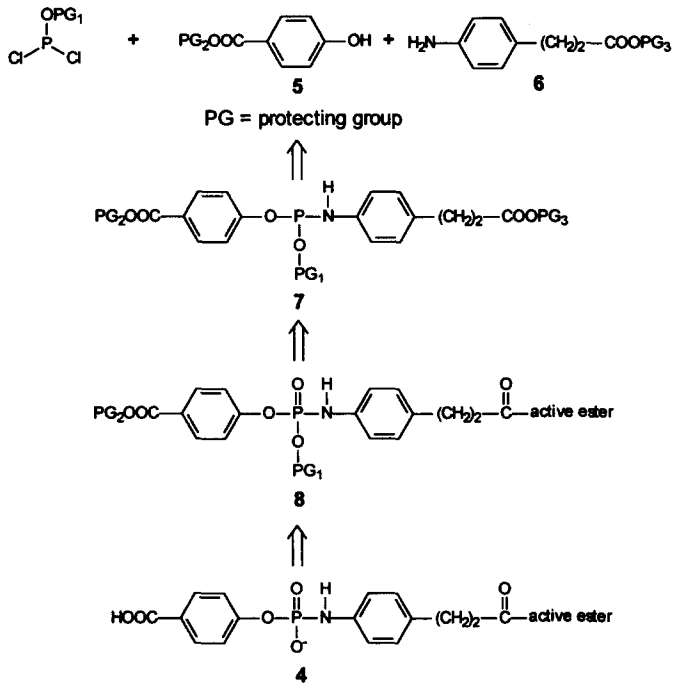


Scheme 2

problematic when the aryl rings contain large and reactive functional goups which would be the case when constructing **4** by this route. Indeed, most *N,O*-arylphosphoramidates synthesized to date have relatively small and unreactive groups attached to the aryl rings or none at all.[14] Ideally, we wished to develop a methodology by which *N,O*-arylphosphoramidates could be constructed without having to isolate any activated species such as *N*-arylphosphoramidic dichloridates or *O*-arylphosphoric dichloridates. Consequently, tricoordinate phosphite chemistry, which has found extensive use in the synthesis of complex organophosphorus compounds, was examined as a means for synthesizing *N,O*-arylphosphoramidates. Herein, we report that *O*-alkyl *N,O'*-aryl phosphoramidates can be synthesized in a one pot procedure by reacting phenol and aniline derivatives with alkyldichlorophosphites to form phosphoramidites followed by oxidation with *m*-CPBA. Selective cleavage of the alkyl group under mild, neutral conditions affords the corresponding *N,O*-arylphosphoramidates. This methodology was successfully applied to the synthesis of **4**.

RESULTS AND DISCUSSION

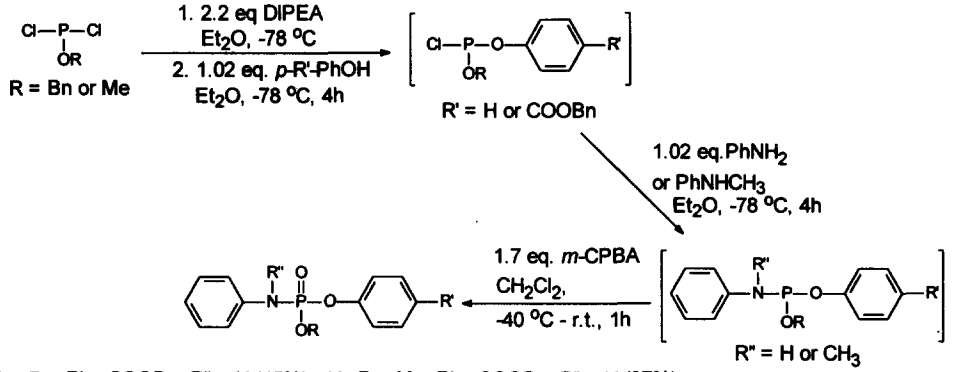
To our knowledge, *N,O*-arylphosphoramidates have never been synthesized using phosphite chemistry. However, this approach has been used for the synthesis of other types of phosphoramidates such as *N,O*-alkylphosphoramidates.[14] One of the more recent and successful examples of the synthesis of complex phosphoramidates using a phosphite approach was described by Martin and coworkers.[16,17] In this instance, two *N,O*-alkylphosphoramidate phospholipid analogues were prepared in yields of 40% and 78% by reacting methyldichlorophosphite consecutively with 2.2 eq. Hunig's base at –78 °C in THF followed by the addition of 1 eq. of a primary alcohol and 1 eq. primary alkylamine followed by oxidation with hydrogen peroxide. We reasoned that this approach would be applicable to the synthesis of **4** by coupling suitably protected phenol (**5**) and aniline derivatives (**6**) with an alkyldichlorophosphite (Scheme 3). This would yield phosphoramidite **7** which would then be oxidized to give the completely protected *O*-alkyl *N,O'*-arylphosphoramidate. Deprotection of the aliphatic carboxylic acid and conversion to the activated ester



Scheme 3

group would yield the TSA precursor **8**. **4** would then be obtained by removal of the aromatic carboxylic acid and the phosphoramidic acid protecting groups. The correct choice of protecting groups (PG_1, PG_2 and PG_3 in Scheme 3) would be crucial for the successful synthesis of **4**. Although stable in neutral and mildly basic environments, phosphoramidates are somewhat acid sensitive and so protecting groups that required acidic conditions for removal were avoided. On the other hand, the active ester portion would be expected to be sensitive to even mildly basic conditions and many nucleophiles. Consequently, it was necessary to employ protecting groups that could be manipulated under mild, neutral conditions. In addition, we wished to employ a protecting group strategy that would minimize unnecessary operations. This would involve differentially protecting the two carboxyl groups ($PG_2 \neq PG_3$ in Scheme 3) so that the aliphatic carboxyl could be selectively converted into the active ester and, ideally, having the aromatic carboxyl moiety and the phosphoramidate protected with the same group ($PG_1 = PG_2$ in Scheme 3), such as the benzyl group, so that **4** could be obtained in a one-pot hydrogenolysis reaction at the end of the synthesis.

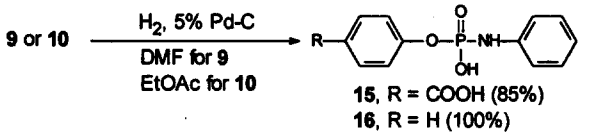
To examine whether the phosphite approach would be applicable to the synthesis of *N,O*-arylphosphoramidates, the synthesis of a series of simple *O*-alkyl *N,O'*-arylphosphoramidate diesters (**9-14**, Scheme 4) was attempted. By converting these species into the *N,O*-arylphosphoramidates, they could be used as truncated TSA's for competition binding assays[18] during antibody screening to identify antibodies that recognize crucial transition state elements. Methyl- or benzyldichlorophosphite was chosen as starting



9, R = Bn, R' = COOBn, R" = H (45%) **12**, R = Me, R' = COOBn, R" = H (37%)
10, R = Bn, R' = H, R" = H (58 %) **13**, R = Me, R' = H, R" = CH_3 (48%)
11, R = Bn, R' = H, R" = CH_3 (40%) **14**, R = Me, R' = COOBn, R" = CH_3 (25%)

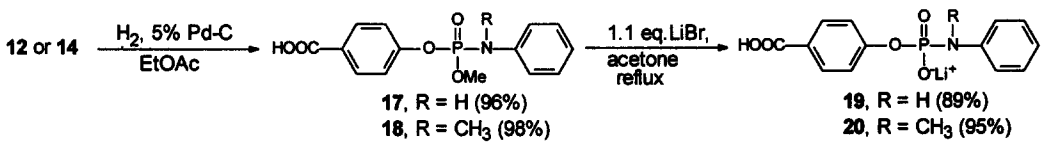
Scheme 4

materials since they are either commercially available (methyl) or can be readily synthesized (benzyl)[19] and removal of methyl or benzyl groups from phosphoramidate esters under mild, neutral conditions via hydrogenolysis[20] or treatment with halide salts[21] has been well documented. By making minor modifications to Martin's procedure,[16,17] **9-14** were constructed in overall yields ranging from 37-58% (Scheme 4) with the exception of **14** which was obtained in a 25 % yield. Addition of the phenolic derivative first followed by the amine worked best and the presence or absence of an *N*-methyl group on the amine did not appear to affect the yields. Methyl- and benzyldichlorophosphite gave similar yields. Consistent with Martin's studies, removal of excess Hunig's base and its hydrochloride salt was necessary for achieving optimal yields on the oxidation reactions. However, although comparable yields were obtained using either THF or ether as solvent, removal of the hydrochloride precipitate was more readily achieved using ether. Non-aqueous conditions for the oxidation reaction using *m*-CPBA in CH_2Cl_2 at -40 °C gave the best yields[22] and the *m*-chlorobenzoic acid produced in the reaction did not appear to affect the yields of the final product. In contrast to Martin's report, significant quantities of symmetrical, disubstituted products resulting from the addition of two phenolic or aniline derivatives to the chlorophosphite were obtained. Although slow addition of the phenol to the chlorophosphite helped alleviate this problem to a small extent, we were unable to find conditions (lower temperatures, alternative solvents, and shorter reaction times were examined) that prevented this from occurring. For those compounds containing benzyl groups at phosphorus, the phosphoramidic acids could be obtained via hydrogenolysis (Scheme 5). The hydrogenolysis (H_2, 5% Pd/C) reaction with compounds containing only one benzyl group, such as **10**, proceeded cleanly in EtOAc in excellent yield, but less so with protic solvents such as methanol. For **9**, which contained both a benzyl-protected carboxyl acid and phosphoramidate acid, only decomposition products were obtained using EtOAc as solvent. This may have been due to the insolubility of the highly polar products in EtOAc which resulted in a high, local concentration of the acidic products and decomposition. However, both benzyl groups in **9** were removed cleanly in a single



Scheme 5

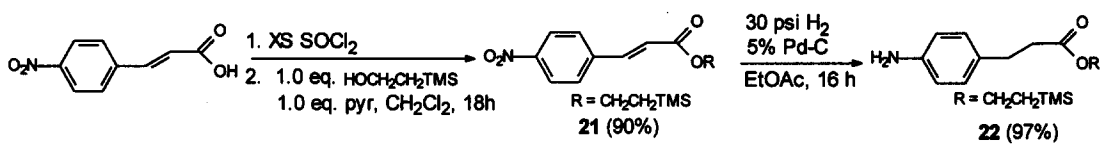
step in near quantitative yield by hydrogenolysis using DMF as solvent (Scheme 5). Attempts to directly obtain the phosphoramidic acids as salts by removing the benzyl group on phosphorus using a halide salt in refluxing acetone or butanone resulted in the formation of a small amount of byproduct resulting from benzylation of the nitrogen in **9** or **10**. However, the phosphoramidic acids could be obtained as pure lithium salts from the *O*-methyl *N,O'*-arylphosphoramidates in good to excellent yields by hydrogenolysis of the



Scheme 6

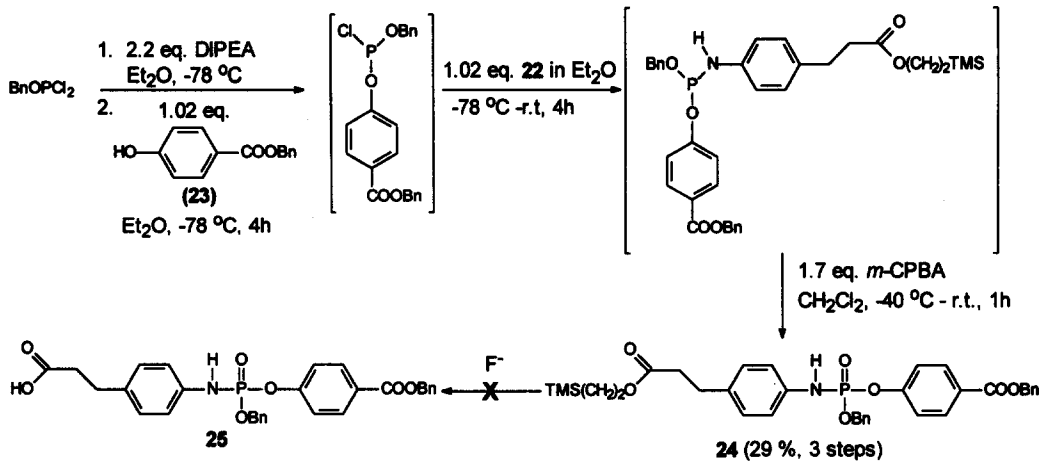
benzyl ester (EtOAc as solvent) followed by treatment with LiBr in refluxing acetone (Scheme 6). We also attempted to remove the methyl groups using TMSBr[23] however, we found that this procedure did not proceed as cleanly as the above reactions.

Although the yields of the *O*-alkyl *N,O'*-arylphosphoramidates (**9-14**) were modest it was apparent that the phosphite methodology would be particularly suited to the synthesis of more complex phosphoramidates such as **4**. Thus, the amino component **22** was prepared (Scheme 7) with the aliphatic carboxyl group protected as a 2-trimethylsilylethyl ester since this group can be removed under mild conditions using



Scheme 7

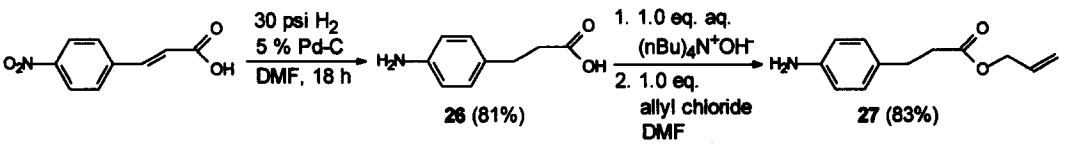
fluoride ion.[24] This was accomplished by converting *p*-nitrocinnamic acid to the acid chloride using thionyl chloride and reacting the crude acid chloride with 2-trimethylsilylethanol to give the ester **21** (90% yield). Hydrogenation of **21** yielded the amine **22** in near quantitative yields (Scheme 7). Benzyldichlorophosphite was reacted with benzyl-protected *p*-carboxyphenol **23** in ether at –78 °C in the presence of Hunig's base followed by the addition of **22** and then oxidation with *m*-CPBA to give the fully protected phosphoramidate **24** in an overall yield of 29% (3 steps, Scheme 8). However, attempts to selectively remove the 2-trimethylsilylethyl protecting group using fluoride ion gave a mixture of products. For example, treating **24**



Scheme 8

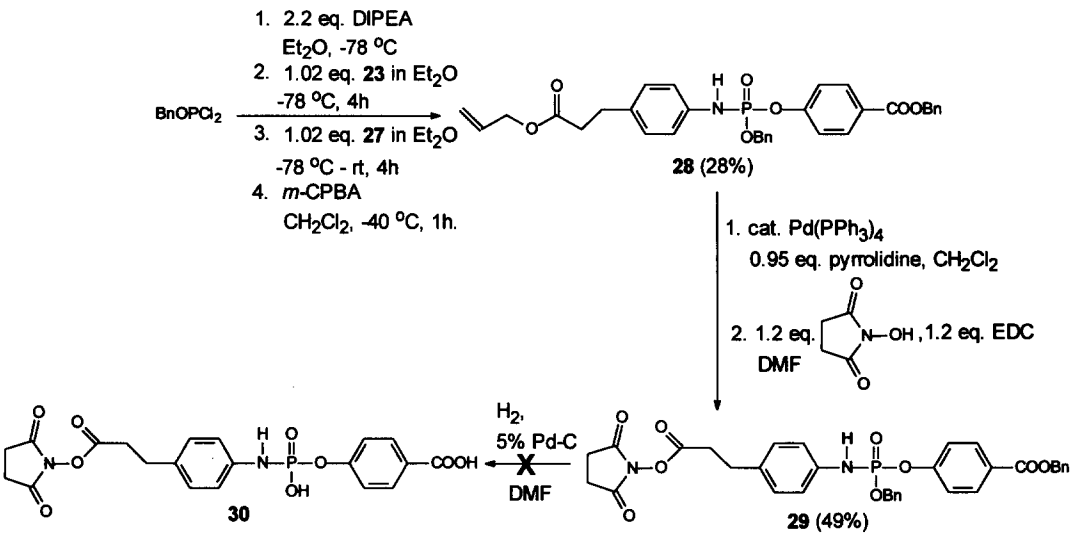
with $(n\text{-}Bu)_4N^+F^-$ in THF resulted not only in loss of the 2-trimethylsilylethyl group but also partial loss of the benzyl protecting group on the phosphate moiety.

Due to the problems encountered with the 2-trimethylsilylethyl group, the aliphatic carboxyl group was protected with an allyl group (**27**, Scheme 9) since this group can be removed under mild, neutral conditions



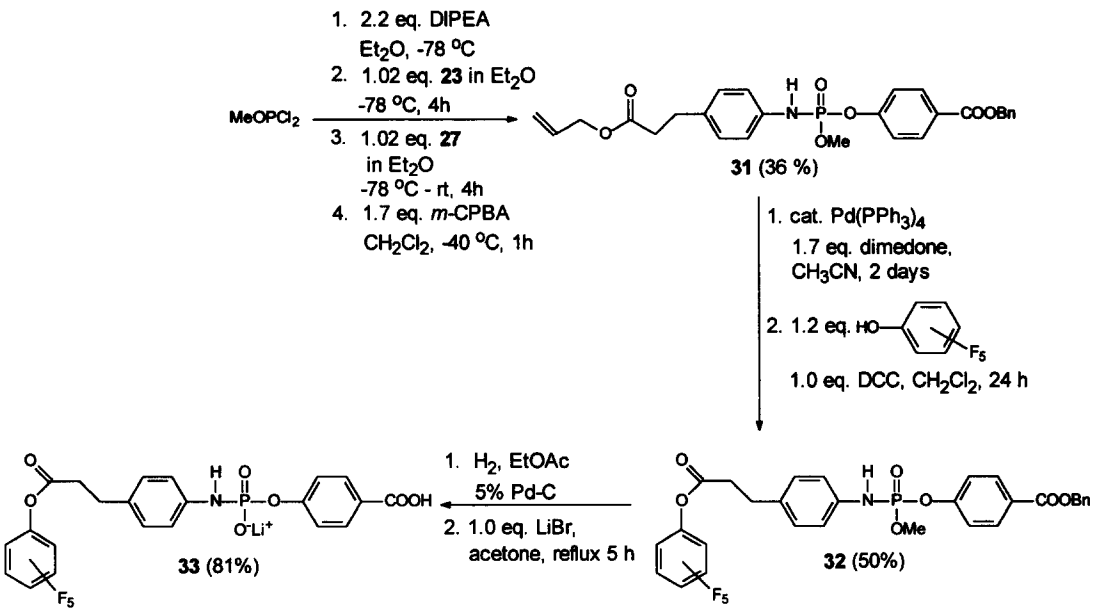
Scheme 9

using a palladium catalyst and a soft nucleophile.[25,26] This was accomplished by hydrogenation of *p*-nitrocinnamic acid to give the amino acid **26**, conversion to the tetrabutylammonium salt followed by reaction with allylchloride in DMF to give the desired allyl ester **27** (overall 67 %, Scheme 9). Using the usual procedure, **23** and **27** were coupled to benzyldichlorophosphite (Scheme 10) to form the phosphoramidate **28**, in an overall yield of 28 %. The allyl group was removed using a catalytic amount of $Pd(PPh_3)_4$ and 1 eq. of pyrrolidine and the resulting crude reaction mixture was filtered and then reacted with N-hydroxysuccinimide (NHS) in the presence of EDC in DMF. This yielded the phosphoramidate diester **29** in which the aliphatic carboxyl group is converted to a reactive NHS ester (49 %, two steps), the ester group most commonly used for forming protein conjugates.[27] However, attempts to obtain the transition state analogue **30** in a single step by mild hydrogenolysis of the benzyl protecting groups using 5% Pd-C under one atm. of H_2 in DMF failed due to decomposition of the phosphoramidate. The NHS esters was also cleaved during the hydrogenolysis reaction. Decomposition of the phosphoramidate may have been a result of the acidic environment created by the presence of two carboxylic acid groups and the phosphoramidic acid group formed during the reaction.



Scheme 10

Due to the difficulties in obtaining **30** as a stable free acid, it was decided to attempt to obtain the TSA as a lithium salt, which we anticipated would be more stable than the free acid, although this would require an additional step. In addition, it was decided that a pentafluorophenyl (Pfp) ester would be a more suitable activated ester since it is stable to hydrogenolysis as opposed to an NHS ester which was unstable to our hydrogenolysis conditions. To our knowledge, Pfp esters have never before been used for forming hapten-protein conjugates. However, it was anticipated that this group would be sufficiently reactive since it has been used extensively for activating amino acids for peptide synthesis.[28] Thus, methyldichlorophosphite was reacted with **23** and **27** in the usual manner followed by oxidation to give phosphoramidate **31** in an overall yield of 36 % (Scheme 11). Prior to the removal of the allyl protecting group on **31**, we performed some studies on model *O*-methyl *N,O'*-arylphosphoramidates in an attempt to improve the yields obtained from the deprotection and coupling reactions. These studies revealed that pyrrolidine, which was used in the deallylation of **28**, also reacts with the methyl protecting group on phosphorus. Although slower than allyl deprotection, demethylation was fast enough to affect the yield of the deallylation reaction. However, we found that dimedone, a nucleophile commonly used for Pd-catalyzed allyl ester deprotection,[29] did not attack the methyl protecting group on phosphorus. Thus, deallylation of **31** was performed with $Pd(PPh_3)_4$ in the presence of excess dimedone and the resulting crude acid was reacted with Pfp using DCC as coupling agent[30] to form the active ester **32**. However, the yield of **32** was not improved compared to that obtained in converting **28** to **29** using pyrrolidine. It is possible that the coupling of Pfp to the free acid proceeds less efficiently than the coupling of NHS to the pyrrolidine salt obtained during the deprotection of **28**. The TSA **33**, was obtained by hydrogenolysis of the benzyl ester in **32** followed by treatment of the crude reaction mixture with LiBr in refluxing acetone. **33** was formed as a lithium salt which precipitated out of solution



Scheme 11

during the reaction. Filtration of the reaction mixture followed by extensive washing of the precipitate with dry acetone yielded pure **33** (81 % two steps). For antibody production, a bovine serum albumin (BSA) conjugate of **33** was prepared by dissolving an excess amount of the **33** in bicarbonate buffer (pH 8.5) containing BSA and stirring at room temperature overnight (11-12 haptens/BSA obtained).

CONCLUSION

A versatile and general procedure for the synthesis of *O*-alkyl *N,O'*-arylphosphoramidates utilizing phosphite chemistry was described. This methodology should be applicable to the synthesis of a wide variety of *O*-alkyl *N,O'*-arylphosphoramidates especially those that are inaccessible by traditional phosphate chemistry. This methodology was used to synthesize an *N,O*-phosphoramidate TSA for carbamate hydrolysis. The TSA was coupled to BSA and a single chain Fv phage display library is currently being screened for antibodies specific for the TSA.[31] High affinity antibodies will be chosen for overexpression and examined for their ability to catalyze the hydrolysis of carbamate-based prodrugs for ADAPT.

ACKNOWLEDGEMENTS

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EXPERIMENTAL

General. Unless otherwise noted, all starting materials were obtained from commercial suppliers (Aldrich or Lancaster) and were used without further purification. Tetrahydrofuran (THF) and diethylether (ether) were distilled form sodium/benzophenone ketyl under argon. CH_2Cl_2 was distilled from calcium hydride under nitrogen. DMF was distilled under reduced pressure from calcium hydride and stored over 4-A sieves under argon. Diisopropylethylamine was distilled over calcium hydride and stored over 4-A molecular sieves under argon. Reactions involving moisture sensitive reagents were executed under an inert atmosphere of dry argon. Liquid transfers were made using oven dried syringes and needles. Flash chromatography was performed using silica gel 60 (Toronto Research Chemicals, 230-400 mesh ASTM). Melting points were obtained on an Electrothermal Inc. melting point apparatus and are uncorrected. ^1H, ^{31}P and ^{19}F NMR spectra were recorded at on a Varian 200-Gemini NMR machine at approximately 200 MHz, 80 MHz and 188 MHz respectively. ^{13}C spectra were recorded on a Varian-500 at approximately 125 MHz unless stated otherwise. For ^1H NMR's run in $CDCl_3$, chemical shifts (δ) are reported in parts per million (ppm) downfield relative to the internal standard tetramethylsilane (TMS). For ^1H NMR spectra run in D_2O, DMSO-d_6, NaOD and CD_3OD, chemical shifts are reported in parts per million relative to the solvents residual protons (D_2O, δ 4.68; DMSO-d_6, δ 2.49 for the central peak of the quintet; NaOD δ 4.68; and CD_3OD, δ 3.30 for the central peak of the quintet). For ^{13}C spectra run in $CDCl_3$, DMSO-d_6 and CD_3OD, chemical shifts are reported in parts per million relative to the solvent residual carbons ($CDCl_3$, δ 77.0 for the central peak; DMSO-d_6, δ 39.5 for the central peak; CD_3OD, δ 49.0 for the central peak). For ^{13}C spectra run in NaOD or D_2O, chemical shifts are reported in parts per million relative to *para*-dioxane (δ 67.4, external). For ^{31}P NMR spectra, chemical shifts are reported in parts per million relative to 85% phosphoric acid (external). For ^{19}F NMR, chemical shifts are reported in parts per million relative to trifluoroacetic acid (external). Spectral splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; comp, complex multiplet and broad. Electron impact (EI) and fast atom bombardment (FAB) mass spectra (MS) were obtained on a Micromass 70-S-250 mass spectrometer. Negative ion FABMS were obtained using a glycerol or p-nitrobenzyl alcohol matrix. Positive ion FABMS were obtained using a glycerol matrix.

Benzyldichlorophosphite. Benzyl alcohol (2.38 mL, 23.0 mmol, 1.0 equiv) in dry ether (45 mL) was added dropwise to a solution of PCl_3 (2.62 mL, 30.0 mmol, 1.3 equiv) in dry ether (25mL) at -40°C over a period of 1 h. After stirring for 1 h, the mixture was warmed at room temperature and distilled under reduced pressure to give the product (4.15 g, 86%), b. p. 65-67 °C/200-220μ (lit[33]: 65 °C/200μ). ^1H NMR ($CDCl_3$) δ: 7.43 (s, 5H), 5.28 (d, 2H, J_{PH} = 8.0 Hz); ^{31}P NMR ($CDCl_3$): δ 175.70.

General procedure for the preparation of O-benzyl- or O-methyl- O'-aryl N-arylphosphoramidates. Dry ether (7 mL) was added to a 100 mL 3-neck round bottom flask containing benzyl- or methyl-

dichlorophosphite (2.39 mmol, 1.0 equiv) and the solution was cooled to –78 °C using a dry ice/acetone bath. A solution of di*iso*propylethylamine (DIPEA, 0.92 mL, 5.26 mmol, 2.2 equiv) in dry ether (5 mL) was added via syringe over a period of 3 minutes to the stirred phosphite solution and stirred for 10 minutes. A solution of the phenol derivative (2.43 mmol, 1.02 equiv) in dry ether (5 mL) was added over a 15-30 minute period and the solution was stirred for 4 h. A solution of the aniline derivative (2.43 mmol, 1.02 equiv) in dry ether (5 mL) was added over a 15-30 minute period and the solution was stirred for 3-4 h. The solution was allowed to warm to room temperature and stirred for 1 h. The solid was filtered through a sintered glass funnel, the filtrate was concentrated by rotary evaporation and the residue was pumped down under high vacuum for 15 minutes. The residual oil was dissolved in dry CH_2Cl_2 (10 mL) and cooled to -40°C. A solution of *m*-CPBA (0.70 g, 4.06 mmol, 1.7 equiv) in dry CH_2Cl_2 (10 mL) was added over a period of 10 minutes during which time a precipitate formed. The solution was allowed to warm to room temperature (the precipitate dissolved) and stirred for 1 h. The mixture was cooled back to -40°C, the precipitate reappeared, and the cold solution was rapidly filtered through a sintered funnel. The filtrate was transferred to an Erlenmeyer flask and a 5% sodium sulfite solution (20 mL) was added and the mixture stirred vigorously. The organic layer was separated, washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$) and concentrated by rotary evaporation yielding a yellowish oil. Pure product was obtained by silica gel flash chromatography.

O-Benzyl O'-(4-benzyloxycarbonyl)phenyl N-phenylphosphoramidate (**9**). Obtained as a pale yellow oil (45%) from benzyl dichlorophosphite, 4-(hydroxy)benzoic acid benzyl ester (**23**) and aniline following the general procedure for the preparation of phosphoramidates. TLC (40% EtOAc/60% hexane): R_f = 0.30; ^{1}H NMR ($CDCl_3$): δ 8.00 (d, 2H, J = 8.4 Hz), 7.78 (d, 1H, J_{PH} = 10.0 Hz), 7.27-7.45 (m, 14H), 7.18 (d, 2H, J = 8.4 Hz), 7.05 (t, 1H, J = 6.0 Hz), 5.36 (s, 2H), 5.16-5.36 (m, 2H); ^{31}P NMR ($CDCl_3$): δ -3.64; ^{13}C NMR ($CDCl_3$): δ 165.5, 154.0 (d, J_{CP} = 5.8 Hz), 138.8, 135.9, 135.1 (d, J_{CP} = 7.8 Hz), 131.6, 129.3, 128.7, 128.6, 128.3, 128.1, 126.9, 122.2, 120.3 (d, J_{CP} = 5.9 Hz), 117.8 (d, J_{CP} = 7.8 Hz); MS (EI): *m/z* 473 (M^+), 382 (M^+ - Bn), 366 (M^+ - OBn), 301, 121, 91(Bn); HRMS: calcd for $C_{27}H_{24}NO_5P$ (M^+) *m/z* 473.1392, found 473.1385.

O-Benzyl O'-phenyl N-phenylphosphoramidate (**10**). Obtained as a white solid (58%) from benzyl dichlorophosphite, phenol and aniline following the general procedure for the preparation of phosphoramidate. MP: 114-114.5; TLC (40% EtOAc/60% hexane): R_f = 0.40 ; ^{1}H NMR ($CDCl_3$): δ 7.07-7.33 (m, 15H), 6.80 (s, 1H), 5.16 (m, 2H); ^{31}P NMR ($CDCl_3$): δ -3.83; ^{13}C NMR ($CDCl_3$): δ 150.2 (d, J_{CP} = 6.0 Hz), 139.2, 135.4 (d, J_{CP} = 7.7 Hz), 129.6, 129.2, 128.44, 128.46 125.1, 121.8, 120.4 (d, J_{CP} = 4.2 Hz), 117.8 (d, J_{CP} = 7.7 Hz), 64.5 (d, J_{CP} = 3.4 Hz); MS (EI): *m/z* 339 (M^+), 182 (M^+ - OPh), 167 ((M^+ - OPh) – Ph), 91 (Bn); HRMS: calcd for $C_{19}H_{18}NO_3P$ *m/z* 339.1024, found 339.1032.

O-Benzyl O'-phenyl N-methyl-N-phenylphosphoramidate (**11**). Obtained as a pale yellow oil (40%) from benzyl dichlorophosphite, phenol and *N*-methylaniline following the general procedure for the

preparation of phosphoramidates. TLC (50% EtOAc/60% hexane): R_f = 0.65; ^{1}H NMR ($CDCl_3$): δ 7.10-7.35 (m, 15H), 5.19 (m, 2H), 3.21 (d, 3H, J_{PH} = 9.2 Hz); ^{31}P NMR ($CDCl_3$): δ -0.57; ^{13}C NMR ($CDCl_3$): δ 150.7 (d, J_{CP} = 6.8 Hz), 143.6 (d, J_{CP} = 4.9 Hz), 135.7 (d, J_{CP} = 6.9 Hz), 129.6, 129.0, 128.5, 128.4, 128.0, 124.8, 124.3, 122.9 (d, J_{CP} = 3.9 Hz), 120.2 (d, J_{CP} = 4.9 Hz), 68.7 (d, J_{CP} = 4.9 Hz), 37.3; MS (EI): *m/z* 353 (M^+), 262 (M^+ - Bn), 167, 91(Bn); HRMS: calcd for $C_{20}H_{20}NO_3P$ *m/z* 353.1181, found 353.1186.

O-Methyl O'-(4-benzyloxycarbonyl)phenyl N-phenylphosphoramidate (**12**). Obtained as a pale yellow oil (37%) from methyl dichlorophosphite, **23** and aniline following the general procedure for the preparation of phosphoramidates. TLC (40% EtOAc/60% hexane): R_f = 0.25; ^{1}H NMR ($CDCl_3$): δ 7.99 (d, 2H, J = 8.6 Hz), 7.75 (d, 1H, J_{PH} = 16.0 Hz), 7.02-7.44 (m, 12H), 5.35 (s, 2H), 3.87 (d, 3H, J_{PH} = 11.7 Hz); ^{31}P NMR ($CDCl_3$): δ -2.24. ^{13}C NMR ($CDCl_3$): δ 165.4, 153.9 (d, J_{CP} = 5.8 Hz), 138.5, 135.8, 131.6, 129.4, 128.5, 128.2, 128.1, 126.9, 122.4, 120.2 (d, J_{CP} = 4.9 Hz), 117.7 (d, J_{CP} = 6.8 Hz), 66.7, 53.7 (d, J_{CP} = 4.9 Hz); MS (EI): *m/z* 397 (M^+), 290 (M^+- OBn), 91 (Bn); HRMS: calcd for $C_{21}H_{20}NO_5P$ *m/z* 397.1079, found 397.1066.

O-Methyl O'-phenyl N-methyl-N-phenylphosphoramidate (**13**). Obtained as a pale light brown oil (48%) from methyl dichlorophosphite, phenol and *N*-methylaniline following the general procedure for the preparation of phosphoramidates. TLC (40% EtOAc/60% hexane): R_f= 0.39; ^{1}H NMR ($CDCl_3$): δ 7.11-7.19 (m, 5H), 7.25-7.32 (m, 5H), 3.82 (d, 3H, J_{PH} = 11.4 Hz), 3.23 (d, 3H, J_{PH} = 9.2 Hz); ^{31}P NMR ($CDCl_3$): δ 0.62; ^{13}C NMR ($CDCl_3$): δ 150.6 (d, J_{CP} = 6.8 Hz), 143.5 (d, J_{CP} = 4.9 Hz), 129.6, 129.0, 124.8, 124.4, (d, J_{CP} = 3.9 Hz), 120.1 (d, J_{CP} = 4.9 Hz), 53.6 (d, J_{CP} = 5.9 Hz), 37.4 (d, J_{CP} = 4.9 Hz); MS (EI): *m/z* 277 (M^+), 184 (M^+ - OPh), 106 (MeNPh); HRMS: calcd for $C_{14}H_{16}NO_3P$ *m/z* 277.0868, found 277.0861.

O-Methyl O'-(4-benzoxycarbonyl)phenyl N-methyl-N-phenylphosphoramidate (**14**). Obtained as a pale yellow oil (25%) from methyl dichlorophosphite, **23**, and *N*-methylaniline following the general procedure for the preparation of phosphoramidates. TLC (50% EtOAc/50% hexane): R_f = 0.33; ^{1}H NMR ($CDCl_3$): δ 8.05 (d, 2H, J = 8.8 Hz), 7.10-7.43 (m, 12H), 5.36 (s, 2H), 3.86 (d, 3H, J_{PH} = 11.7 Hz), 3.24 (d, 3H, J_{PH} = 9.2 Hz); ^{31}P NMR ($CDCl_3$): δ 0.38; ^{13}C NMR ($CDCl_3$): δ 165.5, 154.5 (d, J_{CP} = 6.8 Hz), 143.2 (d, J_{CP} = 3.9 Hz), 135.9, 131.6, 129.1, 128.6, 128.3, 128.2, 126.6, 124.8, 123.1 (d, J_{CP} = 3.9 Hz), 119.9 (d, J_{CP} = 4.8 Hz), 66.7 (s), 53.8 (d, J_{CP} = 5.8 Hz), 37.5 (d, J_{CP} = 4.9 Hz); MS (EI): *m/z* 411 (M^+), 304 (M^+ - OBn), 106 (MeNPh), 91 (Bn); HRMS: calcd for $C_{22}H_{22}NO_5P$ *m/z* 411.1236 (M^+), found 411.1232.

O-(4-Carboxyphenyl)-N-phenylphosphoramidic acid (**15**). 5% Pd/C (16 mg) was added to a solution of **9** (161 mg, 0.363 mmol) in DMF (3mL). The flask was flushed with hydrogen, a balloon filled with hydrogen was fixed onto the flask and the mixture was stirred overnight. TLC showed all the starting material had reacted. The reaction was transferred to two Eppendorf 1.5 mL microcentrifuge tubes and centrifuged for 10 minutes to remove catalyst. The supernatant was removed and concentrated to give an oil which was triturated with chloroform (5 mL) to deposit **15** as a pale yellow solid (90 mg, 85% yield). MP: 142-143 °C; ^{1}H NMR (CD_3OD): δ 7.98 (d, 2H, J = 8.4 Hz), 7.09-7.47 (m, 6H), 6.90 (t, 1H, J = 7.1 Hz); ^{31}P NMR

(DMSO-d_6): δ -1.41; ^{13}C NMR (DMSO-d_6): δ 166.7, 155.0 (d, J_{CP} = 6.7 Hz), 141.4, 131.1, 128.9, 126.4, 120.3, 120.1 (d, J_{CP} = 4.8 Hz), 117.2 (d, J_{CP} = 7.6 Hz); FABMS (negative ion): *m/z* 292 (M - H)$^-$, 275 (M – H - OH), 153.

O-Phenyl N-phenylphosphoramidic acid (**16**). 5% Pd/C (30 mg) was added to a solution of **10** (300 mg, 0.885 mmol) in EtOAc (20 mL). The flask was flushed with hydrogen gas and a balloon filled with hydrogen was fixed onto the flask and the reaction followed by TLC. One hour later, TLC showed all the starting material had reacted. The catalyst was removed by filtration through filter paper and the solvent was evaporated to give **16** as a white solid (220 mg, 100%). MP: 128-128.5 (lit[34]: 128-130 °C); ^{1}H NMR (CD_3OD): δ 7.12-7.24 (m, 9H), 6.91 (dt, 1H, J = 7.1 Hz, J = 1.0 Hz); ^{31}P NMR (CD_3OD): δ -0.57; ^{13}C NMR (50 MHz, CD_3OD): δ 152.3, 142.1, 130.5, 130.1, 125.7, 122.4, 121.7 (d, J_{CP} = 3.6 Hz), 119.2 (d, J_{CP} = 7.3 Hz); FABMS (negative ion): *m/z* 248 (M – 1)$^-$, 154.

O-Methyl O'-(4-carboxyphenyl) N-phenylphosphoramidate (**17**). 5% Pd/C (35 mg) was added to a solution of **12** (0.390 g, 0.98 mmol) in EtOAc (6 mL). The flask was flushed with hydrogen gas and then fixed a balloon filled with hydrogen. The mixture was stirred overnight. The catalyst was removed by filtration through filter paper and the solvent was evaporated to give **17** as a white solid (0.290 g, 96%). MP: 146.5-147.5; ^{1}H NMR ($CDCl_3$): δ 8.01 (d, 2H, J = 8.8 Hz), 7.20-7.38 (m, 4H), 7.09-7.13 (m, 2H), 6.99 (t, 1H, J = 7.3 Hz), 3.90 (d, 3H, J_{PH} = 11.8 Hz); ^{31}P NMR (CD_3OD): δ 1.77; ^{13}C NMR (CD_3OD): δ 168.0, 154.7 (d, J_{CP} = 6.6 Hz), 139.8, 131.8, 129.4, 128.1, 122.5, 120.4 (d, J_{CP} = 5.3 Hz), 118.5 (d, J_{CP} = 5.9 Hz), 53.6 (d, J_{CP} = 5.9 Hz); MS (EI): *m/z* 307 (M^+) 170 (M^+ - OPhCOOH), 92 (HNPh); HRMS: calc. for $C_{14}H_{14}NO_5P$ *m/z* 307.0604 (M^+), found 307.0609.

O-Methyl O'-(4-carboxyphenyl) N-methyl-N-phenylphosphoramidate (**18**). Obtained as a white solid (0.437 g, 98%) from **14** (570 mg) and 5% Pd/C (55 mg) following the procedure for the preparation of **17**. Purification required column chromatography (10% MeOH/90% CH_2Cl_2). TLC (10% MeOH/90% CH_2Cl_2): R_f = 0.60. MP: 149.5-150.5 °C; ^{1}H NMR ($CDCl_3$): δ 10.50 (s, 1H), 8.05 (d, 2H, J = 8.8 Hz), 7.22-7.32 (m, 7H), 3.88 (d, 3H, J_{PH} = 11.4 Hz), 3.25 (d, 3H, J_{PH} = 9.2 Hz); ^{31}P NMR ($CDCl_3$): δ 0.25; ^{13}C NMR ($CDCl_3$): δ 170.3, 154.8, 143.1 (d, J_{CP} = 3.90 Hz), 132.1, 129.1, 126.3, 124.9, 123.3 (d, J_{CP} = 3.9 Hz), 119.9 (d, J_{CP} = 4.9 Hz), 53.9 (d, J_{CP} = 5.8 Hz), 37.6 (d, J ~ 2.9 Hz); MS (EI): *m/z* 321 (M^+), 184 (M^+ - OPhCOOH); HRMS: calcd for $C_{15}H_{16}NO_5P$ *m/z* 321.0766 (M^+), found 321.0766.

Lithium O-(4-carboxy)phenyl-N-phenylphosphoramidate (**19**). Lithium bromide (54 mg, 0.62 mmol, 1.1 equiv) was added to a solution of **17** (173 mg, 0.56 mmol, 1.0 equiv) in dry acetone (2 mL). The reaction mixture was stirred under reflux for 5 h, during which time a white precipitate formed. The precipitate was collected by filtration, and washed with dry acetone to give **19** as a white powder (150 mg, 89%). ^{1}H NMR (CD_3OD): δ 7.89 (d, 2H, J = 8.4 Hz), 7.12-7.22 (m, 6H), 6.78 (m, 1H); ^{31}P NMR (CD_3OD): δ -3.05; ^{13}C NMR (50 MHz, DMSO-d_6): δ 167.9, 157.6 (d, J_{CP} = 7.3 Hz), 144.2, 130.4, 128.2, 125.6, 119.8, 118.0 (d, J_{CP}

= 4.8 Hz), 116.7 (d, J_{CP} = 5.5 Hz); FABMS: *m/z* 292 $(M - Li^+)^-$, 153.

Lithium O-(4-carboxyl)phenyl N-methyl-N-phenylphosphoramidate (**20**). Obtained as a white solid (0.312 g, 95%) from LiBr (99.5 mg, 1.14 mmol, 1.1 equiv) and **18** (334 mg, 1.04 mmol, 1.0 equiv) in dry acetone (4 mL) following the procedure for the preparation of **19**. ^{1}H NMR (CD_3OD): δ 7.90 (d, 2H, *J* = 8.4 Hz), 7.34 (d, 2H, *J* = 8.4 Hz), 7.16-7.25 (m, 4H), 6.90 (t, 1H, *J* = 7.4 Hz), 3.15 (d, 3H, J_{PH} = 8.4 Hz); ^{31}P NMR (CD_3OD): δ 0.43; ^{13}C NMR (DMSO-d_6): δ 167.2, 158.1 (d, J_{CP} = 5.8 Hz), 147.0 (d, J_{CP} = 4.8 Hz), 130.5, 127.8, 124.1, 119.8 (d, J_{CP} = 4.8 Hz), 118.4, 117.7 (d, J_{CP} = 2.9 Hz), 36.0; FABMS: *m/z* 306 $(M - Li^+)$, 261 $(M - Li^+ - COOH)$.

para-Nitrocinnamic acid, 2-trimethylsilylethyl ester (**21**). To a solution of thionylchloride (10 mL, 137 mmol, 5.6 equiv.) was added p-nitrocinnamic acid (4.7g, 24.3 mmol) and the resulting suspension was heated under reflux for 3 hours during which the mixture became a clear homogeneous solution. The excess thionyl chloride was distilled off leaving the crude acid chloride as a pale yellow solid that was pumped down under high vacuum for several hours. The crude acid chloride was dissolved in dry CH_2Cl_2 (50 mL). To this was added a solution of 2-trimethylsilylethanol (3.47 mL, 24.3 mmol, 1 equiv.) and pyridine (2.0 mL, 24.3 mmol, 1 equiv.) in dry CH_2Cl_2 (20 mL) over a period of 20 minutes. The solution was stirred at room temperature for 18 hours. The solution was diluted with 200 mL ether, filtered and the filtrate was concentrated by rotary evaporation. The residue was dissolved in 300 mL ether and washed with 0.1 N HCl (3 x 100 mL), 5 % $NaHCO_3$ (3 x 100 mL), saturated brine (1 x 100 mL), dried ($MgSO_4$) and concentrated by rotary evaporation leaving a pale yellow solid. Pure **21** was obtained by recrystallization from hexane (6.4 g, 90%). ^{1}H NMR ($CDCl_3$): δ 8.25 (d, 2H, *J* = 9.7 Hz), 7.62-7.50 (m, 3H), 6.54 (d, 1H, *J* = 16.2 Hz), 4.28-4.37 (m, 2H), 1.04-1.12 (m, 2H), 0.08 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 166.0, 148.6, 141.3, 140.7, 128.5, 124.1, 123.0, 63.1, 17.5, -1.5; MS (EI): *m/z* 293 (M^+), 263 $(M^+ - 2(CH_3))$, 250, 220 $(M^+ + 1 - Si(CH_3)_3)$, 176 $((M^+ + 1 - CH_2CH_2Si(CH_3)_3)$, 146 $(M^+ - O_2NPhCHCH)$; HRMS: calcd for $C_{14}H_{19}NO_4Si$ *m/z* 293.1083 (M^+), found 293.1091.

3-(4-Aminophenyl)propionic acid, 2-trimethylsilylethyl ester (**22**). 5% Pd/C (500 mg) was added to a solution of **21** (5.9 g, 20.1 mmol) in EtOAc (50 mL) and the resulting mixture subjected to 30 psi hydrogen with vigorous shaking using a Parr hydrogenation apparatus for 16 hours. The solution was filtered through Celite and the filtrate concentrated leaving a yellow oil. The oil was dissolved in 300 mL ether and washed with 5 % $NaHCO_3$ (2 x 150 mL), brine (1 x 150 mL), dried ($MgSO_4$) then concentrated leaving **22** as a pale yellow oil which required no further purification (5.15 g, 97 %). ^{1}H NMR ($CDCl_3$): δ 6.99 (d, 2H, *J* = 8.4 Hz), 6.62 (d, 2H, J = 8.4 Hz), 4.12-4.20 (m, 2H), 3.57 (broad s, 2H), 2.84 (t, 2H, J = 7.0 Hz), 2.50-2.58 (m, 2H), 0.93-1.02 (m, 2H), 0.042 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 173.1, 145.0, 130.5, 129.1, 115.3, 62.4, 36.6, 30.3, 17.5, -1.42; MS (EI): *m/z* 265 (M^+), 237 $(M^+ + 1 - 2(CH_3))$, 164 $(M^+ + 1 - CH_2CH_2Si(CH_3)_3)$, 119, 106 (O_2NPhCH_2), 73; HRMS: calcd for $C_{14}H_{23}NO_2Si$ (M^+) *m/z* 265.1499, found 265.1498.

4-Hydroxybenzoic acid, benzyl ester (**23**). 4-Hydroxybenzoic acid (6.0 g, 43.5 mmol, 1.0 equiv) was dissolved in MeOH-water (1:1, 40 mL). To this was added a 40% tetrabutyl ammonium hydroxide (28.2 mL, 43.5 mmol, 1.0 equiv) solution. After stirring for 2 h, the homogeneous solution was concentrated by rotary evaporator and the residue was pumped down under high vacuum to give a white solid. Benzyl bromide (5.17 mL, 43.5 mmol, 1.0 equiv) was added to the crude salt dissolved in dry DMF (100 mL) over a period of 2 minutes. The mixture was stirred for 2 h and the solvent was removed by rotary evaporator. Water (200 mL) was added, followed by ethyl acetate (200 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 x 200 mL). The combined extracts were washed with 0.5 M hydrochloric acid (200 mL), 5% $NaHCO_3$ (200 mL) and brine (200 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue subjected to column chromatography (silica, 30% EtOAc/70% hexane) to give **23** as a white solid (2.47 g, 86%). mp = 108-109.5 °C (lit[35]: 109-111 °C); TLC (30% EtOAc/70% hexane): R_f = 0.25; ^{1}H NMR ($CDCl_3$): δ 8.00 (d, 2H, J = 8.8 Hz), 7.37-7.47 (m, 5H), 7.12 (s, 1H), 6.91 (d, 2H, J = 8.8 Hz), 5.38 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 166.6, 160.2, 136.0, 132.1, 128.6, 128.2, 128.1, 122.3, 115.2, 66.4; MS (EI): *m/z* 228 (M^+), 121 (M^+ - OBn), 91 (Bn); HRMS: calcd for $C_{14}H_{12}O_3$ *m/z* 228.0786 (M^+), found 228.0788.

O-Benzyl-O'-(4-benzoxycarbonyl)phenyl-N-[4-(2-(trimethylsilylethoxycarbonyl)ethyl)phenyl]-phosphoramidate (**24**). Obtained as a pale yellow oil (29%) from benzyldichlorophosphite, **22** and **23** following the general procedure for the preparation of phosphoramidates. TLC (40% EtOAc/60% hexane): R_f = 0.40; ^{1}H NMR ($CDCl_3$): δ 7.96 (d, 2H, J = 8.4 Hz), 6.95-7.42 (m, 16H), 6.83 (d, 1H, J_{HP} = 6.8 Hz), 5.32 (s, 2H), 5.04-5.25 (m, 2H), 4.14-4.22 (m, 2H), 2.89 (t, 2H, J = 7.4 Hz), 2.57 (t, 2H, J = 7.4 Hz), 0.94-1.02 (m, 2H), 0.044 (s, 9H); ^{31}P NMR ($CDCl_3$): δ -4.11; ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.9, 165.4, 154.4, 137.4, 136.2, 135.5, 134.7, 131.6, 129.2, 128.6, 128.1, 127.1, 120.4, 118.5, 69.0, 66.7, 62.6, 36.2, 30.3, 17.5, -1.4; MS (EI): *m/z* 646 (M^+ + 1), 554, 464, 345, 196, 91; HRMS: calcd for $C_{34}H_{40}NO_7PSi$ *m/z* 645.2312, found 645.2336

3-(4-Aminophenyl)propionic acid (**26**). 5% Pd/C (0.60 g) was added to a solution of 4-nitrocinnamic acid (6.0 g, 31.1 mmol) in DMF (300 mL) and then subjected to 30 psi hydrogen gas with vigorous shaking using a Parr hydrogenation apparatus for 18 hours. DMF was removed by rotary evaporation and EtOAc-MeOH (4:1, 250 mL) was added to the residue. The catalyst was removed by filtration through filter paper and the filtrate was passed through celite. The pale yellow solution was concentrated to give a solid which was recrystallized from EtOAc/$CHCl_3$ (1:2) yielding **26** as a white solid (4.15 g, 81%). MP: 130-131.5 °C (lit[36]: 130-132 °C); ^{1}H NMR (0.1N NaOD): δ 7.09 (d, 2H, J = 8.4 Hz), 6.77 (d, 2H, J = 8.4 Hz), 2.76 (t, 2H, J = 7.2 Hz), 2.40 (t, 2H, J = 7.2 Hz).

3-(4-Aminophenyl)propionic acid allyl ester (**27**). A 40% aqueous solution of tetra-*n*-butylammonium hydroxide (16.20 mL, 25.01 mmol, 1.0 equiv) was added to **26** (4.03 g, 25.01 mmol, 1.0 equiv) in distilled

water (15 mL) and the mixture was stirred until all of **26** had dissolved. The water was evaporated by high vacuum rotary evaporator and the residue was pumped down under high vacuum for several hours to give the tetra-*n*-butylammonium salt of **26** as a white solid (10.01 g) in a near quantitative yield. To a solution of the salt (4.74 g, 11.8 mmol, 1.0 equiv) in dry DMF (20 mL), was added allyl chloride (0.954 mL, 11.7 mmol, 1.0 equiv) in DMF (20 mL) over a period of 2 hours. The solution was stirred for 4h and the solvent was then removed by high vacuum rotary evaporation. The residue was poured into water (250 mL) and the product was extracted with EtOAc (3x150 mL). The combined extracts were washed with 5% aq. $NaHCO_3$, dried ($MgSO_4$) and concentrated and the residue purified by column chromatography (silica, 40% EtOAc/60% hexane) to give **27** as a pale yellow oil (1.98 g, 83%). TLC (40% EtOAc/60% hexane): R_f = 0.60; ^{1}H NMR ($CDCl_3$): δ 6.70 (d, 2H, J = 8.5 Hz), 6.62 (d, 2H, J = 8.5 Hz), 5.81-6.00 (m, 1H), 5.29 (dd, 1H, J = 17.2 Hz, J = 1.60 Hz), 5.23 (dd, J = 10.5 Hz, J = 0.8 Hz), 4.59 (d, 2H, J = 5.5 Hz), 3.59 (s, 2H), 2.87 (t, 2H, J = 7.7 Hz), 2.62 (t, 2H, J = 7.7 Hz); ^{13}C NMR ($CDCl_3$): δ 172.7, 144.6, 132.1, 130.3, 129.0, 118.1, 115.2, 65.0, 36.2, 30.1; MS (EI): *m/z* 205 (M^+), 164 (M^+ - allyl), 106 $(H_2NPhCH_2)^+$; HRMS: calcd for $C_{12}H_{15}NO_2$ *m/z* 205.1103 (M^+), found 205.1111.

O-Benzyl O'-(4-benzoxycarbonyl)phenyl N-[4-(2-(alloxycarbonyl)ethyl)phenyl]phosphoramidate (**28**). Obtained as a pale yellow oil (28%) from benzyl dichlorophosphite, **23** and **27** following the general procedure for the preparation of phosphoramidates. TLC (50% EtOAc/50% hexane): R_f = 0.40; ^{1}H NMR ($CDCl_3$): δ 7.98 (d, 2H, J = 8.4 Hz), 6.95-7.42 (m, 16H), 6.87 (d, 2H, J = 10.3 Hz), 5.81-5.97 (m, 1H), 5.32 (s, 2H), 5.04-5.34 (m, 4H), 4.60 (m, 2H), 2.91(t, 2H, J = 7.5 Hz), 2.62 (t, 2H, J = 7.5 Hz); ^{31}P NMR ($CDCl_3$): δ -4.03; ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.4, 165.5, 154.4, 136.1, 134.6, 132.4, 131.6, 129.2, 128.6, 128.1, 127.6, 127.2, 120.4, 118.5, 118.1, 69.1, 66.7, 65.1, 36.0, 30.3; MS (EI): *m/z* 585 (M^+), 494 (M^+ - Bn), 436 (M^+ - Bn – Oallyl), 91 (Bn); HRMS: calcd for $C_{27}H_{28}NO_7P$ *m/z* 509.1927 (M^+), found 509.1916.

O-Benzyl O'-(4-benzoxycarbonyl)phenyl N-[4-(2-(succinimidylcarbonyl)ethyl)phenyl]phosphoramidate (**29**). To a solution of **28** (100 mg, 0.174 mmol, 1.0 equiv) in dry CH_2Cl_2 (1 mL), was added pyrrolidine (0.0135 mL, 1.65 mmol, 0.95 equiv), $P(Ph)_3$ (6.6 mg, 15 mol%) and $(P(Ph)_3)_4Pd^0$ (14.8 mg, 7.5 mol%). The reaction mixture was stirred at rt. under argon for 2 h and the solvent was evaporated off. The residue was pumped down under high vacuum for 2 h, and redissolved in dry DMF (0.50 mL). N-hydroxysuccinimide (23.6 mg, 0.209 mmol, 1.2 equiv) and EDC (39 mg, 0.209 mmol, 1.2 equiv) was added and the solution was stirred for 16 h. The reaction was filtered and concentrated by rotary evaporation. Column chromatography (silica, 30% EtOAc/70% hexane) of the crude residue yielded pure **29** as an off-white solid (49%). TLC (30% EtOAc/70% hexane): R_f = 0.44; ^{1}H NMR ($CDCl_3$): δ 7.96 (d, 2H, J = 8.4 Hz), 6.95-7.45 (m, 16H), 6.67 (d, 2H, J = 10.3 Hz), 5.81-5.97 (m, 1H), 5.32 (s, 2H), 5.04-5.26 (m, 2H), 2.80-3.05 (m, 4H), 2.79 (s, 4H); ^{31}P NMR ($CDCl_3$): δ -4.32; ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.9, 167.8, 165.6, 154.2, 137.3, 136.1, 135.3, 133.3, 131.6, 129.2, 128.6, 128.1, 127.1, 120.4, 118.6, 69.1, 66.7, 39.0, 36.5, 32.8, 29.8, 25.6; MS (EI): *m/z* 642

(M^+), 436 (M^+ - OBn – $N(COCH_2)_2$), 196, 121, 91 (Bn); HRMS: calcd for $C_{27}H_{28}NO_7P$ (M^+) *m/z* 642.1740, found 642.1767.

O-Methyl O'-[(4-benzoxycarbonyl)phenyl] N-[4-(2-(alloxycarbonyl)ethyl)phenyl]phosphoramidate (**31**). Obtained as a pale yellow oil (36%) from methyl dichlorophosphite, **23** and **27** following the general procedure for the preparation of phosphoramidates. TLC (40% EtOAc/60% hexane) R_f = 0.20; ^{1}H NMR ($CDCl_3$): δ 7.98 (d, 2H, J = 8.8 Hz), 7.36 (m, 5H), 7.16 (d, 2H, J = 8.8 Hz), 7.11 (d, 2H, J = 8.6 Hz), 6.92 (d, 2H, J = 8.6 Hz), 6.05 (d, 1H, J_{PH} = 9.8 Hz), 5.80-6.0 (m, 1H), 5.33 (s, 2H), 5.28 (d, 1H, J = 17.0 Hz), 5.22 (d, 1H, J = 10.5 Hz), 4.58 (dd, 2H, J = 5.8 Hz, J = 0.8 Hz), 3.85 (d, 3H, J_{PH} = 11.8 Hz), 2.91(t, 2H, J = 7.7 Hz), 2.62 (t, 2H, J = 7.7 Hz); ^{31}P NMR ($CDCl_3$): δ -2.92; ^{13}C NMR ($CDCl_3$): δ 172.5, 165.5, 154.0 (d, J_{CP} = 5.9 Hz), 136.9, 135.8, 134.4, 132.1, 131.6, 129.2, 128.6, 128.2, 128.1, 126.9, 120.2 (d, J_{CP} = 4.90 Hz), 118.2, 117.9 (d, J_{CP} = 7.8 Hz), 66.7, 65.1, 53.6 (d, J_{CP} = 4.9 Hz), 35.9, 30.1; MS (EI): *m/z* 509 (M^+), 418 (M^+ - Bn), 360 (M^+ - 1 - OBn – allyl), 91 (Bn); HRMS: calcd for $C_{27}H_{28}NO_7P$ *m/z* 509.1603 (M^+), found 509.1578.

O-Methyl O'-(4-benzoxycarbonyl)phenyl N-[4-(2-(pentafluorophenoxycarbonyl)ethyl)phenyl]phosphoramidate (**32**). To a solution of **31** (0.568 g, 1.12 mmol, 1.0 equiv) in HPLC grade CH_3CN (30 mL), was added dimedone (0.266 g, 1.90 mmol, 1.7 equiv) and $Pd(PPh_3)_4$ (0.129 g, 0.112 mmol, 10 mol%). The reaction mixture was stirred at rt. under argon for 48 h and the solvent was evaporated off. The residue was pumped down under high vacuum for 2 h, and redissolved in dry CH_2Cl_2 (10 mL). Pentafluorophenol (0.227 g, 1.23 mmol, 1.1 equiv) and DCC (0.254 g, 1.23 mmol, 1.1 equiv) was added and the solution was stirred for 24 h. Water (20 mL) was added and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL) and the combined extracts were dried ($MgSO_4$) and concentrated. Column chromatography (silica, 30% acetone/70% hexane) of the crude residue yielded pure **32** as a pale yellow oil (50%). TLC (30% acetone/70% hexane): R_f = 0.44; ^{1}H NMR ($CDCl_3$): δ 8.00 (d, 2H, J = 8.4 Hz), 6.97-7.41 (m, 11H), 5.95 (d, 1H, J_{PH} = 10.0 Hz), 5.33 (s, 2H), 3.88 (d, 3H, J_{PH} = 11.6 Hz), 2.96-3.01 (m, 4H); ^{31}P NMR (CH_2Cl_2 with D_2O insert): δ -0.09; ^{19}F NMR ($CDCl_3$): δ -76.45 (d, 2F, J = 16.9 Hz), -81.80 (d, 1F, J = 18.8 Hz), -86.66 (d, 2F, J = 16.9 Hz); ^{13}C NMR ($CDCl_3$): δ 168.7, 165.5 154.0 (d, J_{CP} = 5.8 Hz), ~134-145 (weak multiplets corresponding to C-F in ArF_5), 137.3, 135.8, 133.2, 131.6, 129.3, 128.6, 128.3, 128.1, 127.0, ~125 (weak multiplet corresponding to O-C in ArF_5),120.2 (d, J_{CP} = 4.8 Hz), 118.0 (d, J_{CP} = 6.7 Hz), 66.7, 53.7 (d, J_{CP} = 4.8 Hz), 35.0, 29.9; MS (EI): *m/z* 635 (M^+), 544 (M^+ - Bn), 452 (M^+ - $OArF_5$), 410 (M^+ - $CH_2COOArF_5$), 91 (Bn); HRMS calcd for $C_{30}H_{23}F_5NO_7P$ *m/z* 635.1132 (M^+), found 635.1120.

Lithium O-(4-Carboxy)phenyl N-[4-(2-(pentafluorophenoxycarbonyl)ethyl)phenyl]phosphoramidate (**33**). A round bottom flask containing **32** (310 mg 0.49 mmol), 5% Pd/C (62 mg) in EtOAc (5 mL) was fitted with a balloon filled with hydrogen and stirred for 5h. The solution was transferred to several 1.5 mL Eppendorf microcentrifuge tubes and centrifuged in an Eppendorf microcentrifuge for 10 minutes. The supernatant was removed and concentrated to give a semi-solid (250 mg). This was dissolved in dry acetone

(5 mL) and heated with LiBr (40 mg, 0.46 mmol) under reflux for 5h. The precipitates were collected by filtration, washed extensively with dry acetone to give **33** as a white powder (212 mg, 81%). ^{1}H NMR (DMSO-d_6): δ 7.77 (d, 2H, J = 7.5 Hz), 7.20 (d, 2H, J = 7.5 Hz), 6.95-7.06 (m, 4H), 6.68 (d, 1H, J_{PH} = 7.6 Hz), 3.15 (broad t, 2H), 2.86 (broad t, 2H); ^{31}P NMR (DMSO-d_6): δ -4.73; ^{19}F NMR (DMSO-d_6): δ -72.85 (d, 2F, J = 19.0 Hz), -77.83 (t, 1F, J = 24.0 Hz), -82.30 (t, 2F, J = 21.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.0, 167.2, 157.8 (d, J_{CP} = 6.6 Hz), 142.0, 130.5, 130.3, 130-140 (weak multiplets corresponding to C-F and C-O in ArF_5), 128.1, 124.2, 119.9 (d, J_{CP} = 5.1 Hz), 116.6 (d, J_{CP} = 17.3 Hz), 35.9, 29.8; FABMS (negative ion): *m/z* 530 (M – Li^+, 100 %), 392 (M – Li^+ - OPhCOOH), 346 (M – Li^+ - $OArF_5$); FABMS (positive ion): m/z 538 (MH^+), HRFABMS (positive ion): calcd for $C_{22}H_{15}F_5NO_7PLi$ (MH^+) *m/z* 538.0674, found 538.0665.

Conjugation of TSAs to bovine serum albumin (BSA). Approximately 3 mg of **33** (~ 5.4 ×10^{-6} moles) and 6 mg of bovine serum albumin (Sigma, 99%, ~ 8.96×10^{-8} moles) were weighed into a vial (~60-fold molar excess of hapten to BSA). Upon addition of 3 mL 50 mM $NaHCO_3$, pH 8.5, followed by gentle shaking, the hapten and BSA were solubilized. The solution was then transferred to a conical centrifuge tube and was shaken gently overnight at room temperature. Unconjugated hapten was removed with Centricon centrifugal concentrators. The remaining hapten-BSA solution in the concentrators was washed with phosphate-buffered saline (PBS: 10 mM KPi, 150 mM NaCl, 0.1% NaN_3, pH 7.5) and again subjected to centrifugation. After repeating the washing procedure three times, the final volume of hapten-BSA conjugate solution (~ 0.2 mL) was diluted to 1 mL with PBS. The BSA concentration was determined to be 0.9 mg/mL, using bicinchoninic acid (BCA method).[37] The number of haptens per BSA was calculated to be between 11 and 12, as determined by the method of Habeeb.[38]

REFERENCES AND NOTES

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