Tartric Acid-Based Linker for the Solid-Phase Synthesis of C-Terminal Peptide α-Oxo Aldehydes

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A novel linker, based on the anchoring of (+)-dimethyl 2,3-*O*-isopropylidene-D-tartrate to PEGA or PEG-PS solid supports, was developed for the solid-phase synthesis of C-terminal peptide α-oxo aldehydes. Peptide elongation was performed using the 9-fluorenylmethoxycarbonyl/*t*-Bu chemistry. The peptide and the 1,2-diol were deprotected on the solid phase. Then, a periodic oxidation of the fully deprotected peptidyl-resin led to the simultaneous cleavage of the product from the solid support and to the generation of the α-oxo aldehyde moiety. The methodology allowed the distance between the α-oxo aldehyde and the peptide to be easily modulated. The C-terminal peptide α-oxo aldehydes synthesized in this study were found to be useful partners in hydrazone, thiazolidine, and oxime chemical ligations.

Introduction

Peptide chemistry possesses efficient and mild methodologies for the site-specific ligation of unprotected peptide fragments in aqueous media. These methods were mainly developed to overcome the difficulties associated with the stepwise synthesis of large structures. Now, proteins or, more generally, high molecular weight constructs can be assembled starting from small peptide fragments, usually synthesized using solid-phase methodologies.

Two fully deprotected peptide fragments can be ligated through an amide bond (native chemical ligation,[1] expressed protein ligation,[2] and pseudoproline ligation[3]), a thioester bond,[4] a thioether bond,[5] a thiazolodine ring[6] or a Schiff base such as an oxime[7] or a hydrazone.[8] More recently, Schmidtchen and co-workers have used the Castro−Stephens−Sonogashira palladium cross-coupling reaction in aqueous media to connect iodoarenes and alkynes.[9,10]

Engaged in a project aimed at synthesizing peptide libraries by thiazolidine, oxime, or hydrazone ligations, we needed a simple and efficient solid-phase methodology for the parallel synthesis of peptides functionalized at the C-terminus by a stable aldehyde moiety. A large amount of work has been devoted to the synthesis of these electrophilic partners.[11] C-Terminal N^{ϵ}-glyoxylyllysyl peptides can be easily obtained by periodic oxidation of the seryl or threonyl precursors in solution.[12] This selective, mild, and high-yielding reaction leads to an α-oxo aldehyde group that is stable over a wide pH range and highly reactive toward 1,2-amino thiols, hydroxylamines, or hydrazines. Analogously, peptides esterified or amidated at the C-terminus with glycerol or 3-amino-1,2-propanediol were oxidized in solution with periodate into glycolaldehydes or glycinals, respectively. For these two approaches, the aldehyde precursor is elaborated on the solid phase, but further chemistry must be performed in solution, an aspect that limits the automation of the process. If we focus on solid-phase methodologies, which are best suited for the parallel synthesis of peptide

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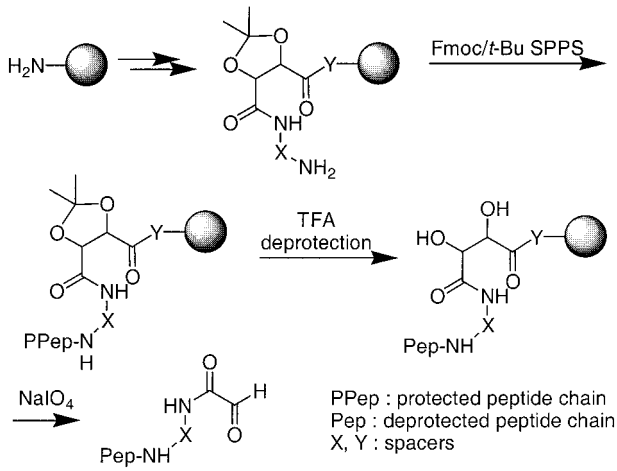
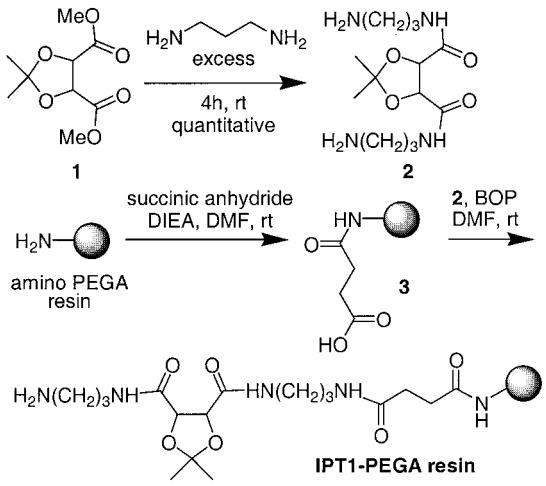
Scheme 1



Scheme 2



libraries, most of them are devoted to the synthesis of peptide aldehydes (PAs), an important class of proteolytic enzyme inhibitors. However, PAs are prone to epimerization, and their synthesis often requires the preparation of α-amino aldehyde derived linkers. Thus, a novel linker was developed that leads to the formation of a C-terminal α-oxo aldehyde function during the separation of the product from the solid support (Scheme 1). It was elaborated starting from (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate, which is commercially available.[13] The isopropylidene moiety was found to be stable during standard Fmoc/*t*-Bu solid-phase peptide synthesis. The side-chain protecting groups and the isopropylidene moiety were removed during a standard acidolytic treatment with concentrated trifluoroacetic acid. The fully deprotected peptidyl resin was subjected to a mild and easily automated periodic oxidation, which led simultaneously to the formation of the α-oxo aldehyde moiety and to the cleavage of the product from the solid support.

We describe in this paper the elaboration of various 2,3-*O*-isopropylidene-D-tartrate based linkers (**IPT** linkers) on PEGA or PEG-PS resins and their use for the synthesis of several C-terminal peptide α-oxo aldehydes. We present also the utility of these compounds aldehydes in oxime, hydrazone, and thiazolidine chemical ligations.

Results and Discussion

Synthesis of the IPT1 Linker: First Approach. The strategy depicted in Scheme 1 required the use of a polymer having good swelling properties in various solvents including water for the final solid-phase periodic oxidation. The poly(ethylene glycol) polyacrylamide copolymer (PEGA) resin fulfilled these requirements and was preferred for the solid-phase chemistry since it was found to be superior to many of the existing polymers for peptide synthesis.[14] One simple way to anchor (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate **1** to the solid support is depicted in Scheme 2. Diamine **2** was synthesized by slow addition of **1** to an excess of 1,3-diaminopropane at room temperature. The excess diamine was removed by evaporation under reduced pressure. Amino PEGA resin was reacted with succinic anhydride to give resin **3**, whose carboxylic acid group was activated with benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent[15]) and reacted with diamine **2** to give the **IPT1**-PEGA resin. The resin was dried and stored at room temperature. The charge was found to be 0.20 mmol/g, as determined by spectrophotometric analysis of the dibenzofulvene−piperidine adduct following derivatization with Fmoc-Gly-OH. **IPT1**-PEGA resin was analyzed by HR-MAS NMR in DMF-d_7. The acetonide appeared as a singlet at 1.40 ppm. The NH region of the TOCSY spectrum, besides the signals at about 7.7 ppm due to the PEGA solid support, displayed three amide protons at 8.28, 8.20 (tartramide NH), and 7.92 ppm (succinamide NH) coupled to *n*-propyl chains.

Peptide Synthesis, Deprotection, and Solid-Phase Periodic Oxidation. The test sequence Ac-KYVS was assembled upon the **IPT1**-PEGA resin using the HBTU/HOBt/DIEA activation and standard Fmoc/*t*-Bu chemistry. The peptidyl-resin was deprotected in a trifluoroacetic acid/anisole/water mixture during 2 h. An HR-MAS NMR spectrum in DMF-d_7 confirmed the complete removal of the side-chain protecting groups and of the acetonide moiety. The fully deprotected Ac-KYVS-**IPT1**-PEGA resin was then treated with an excess of sodium periodate in a water/acetic acid 2/1 mixture containing *p*-nitrophenol as an internal standard. This solvent system, already used by Tam and co-workers for the periodic oxidation of peptide diols in solution, has the advantage of allowing the solubilization of a large variety of peptides.[16] The release of peptide aldehyde **4** (Figure 1) was monitored by reversed-phase HPLC, following the quenching of a small aliquot of the reaction medium with ethylene glycol. The periodic oxidation appeared to be very fast since all the product was released after 30 s of reaction at room temperature. Peptide **4** was isolated with a 38% yield following RP-HPLC purification (Table 1, entry 1).

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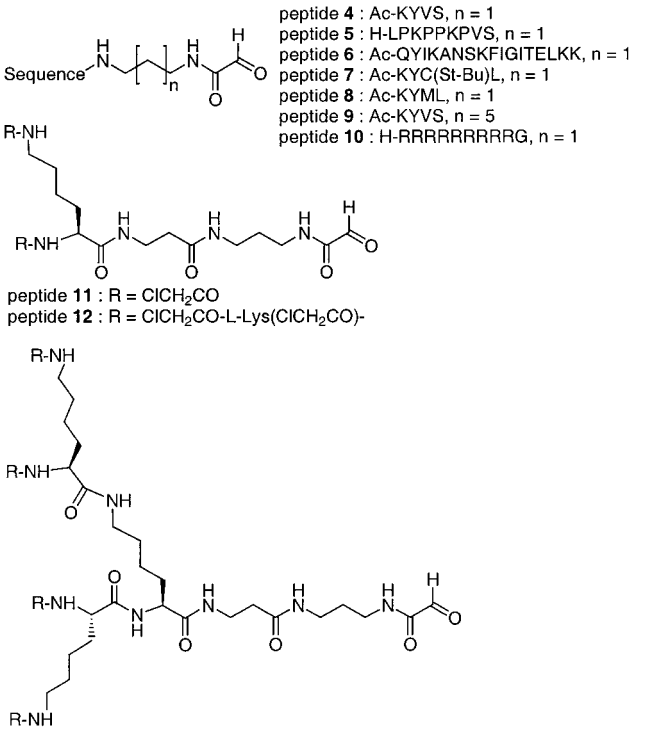


Figure 1. α-Oxo acyl peptides synthesized in this study.

Table 1. Yields of Peptides 4−13

entry	peptide	linker-resin		yield (%)
1	4	IPT1-PEGA	used dry	38.0
2	4	IPT2-Argogel	used dry	38.3
3	4	IPT2-Val-PEGA	used dry	21
4	4	IPT2-Val-PEGA	acylated immediately	58
5	4	IPT2-Novagel	used dry	38.5
6	5	IPT1-PEGA	used dry	26.0
7	6	IPT1-PEGA	used dry	06.1[a]
8	6	IPT2-Val-PEGA	acylated immediately	14.6[a]
9	7	IPT2-PEGA	used dry	52.8
10	8	IPT1-PEGA	used dry	13
11	9	IPT3-PEGA	used dry	31.7
12	11	IPT2-PEGA	used dry	18
13	12	IPT1-PEGA	used dry	31
14	13	IPT2-PEGA	used dry	23.5
15	14	IPT2-PEGA	used dry	21
16	10	IPT2-Val-PEGA	acylated immediately	52

[a] Peptide **6** was dispersed in mannitol before the lyophilization step.

To evaluate the ability of the **IPT** 1-succinyl amino PEGA resin to lead to larger C-terminal peptide α-oxo-aldehydes, the synthesis of peptides **5** and **6** was undertaken. For peptide **5**, a periodic oxidation of 2 min led to a 26% isolated yield following RP-HPLC purification (Table 1, entry 6). Figure 2a corresponds to the RP-HPLC trace of the crude peptide aldehyde **6** released in the periodic oxidation mixture. Peptide **6** was purified as usual using a water/acetonitrile linear gradient. Direct lyophilization of the pure fractions resulted in an aggregated product (Figure 2b). The aggregation was prevented by adding an excess of (+)-D-mannitol to the peptide solution before the lyophilization step (Figure 2c, Table 1, entry 7). Finally, the synthesis of peptide aldehyde **8** was undertaken to check the possibility to cleave the vicinal diol moiety of the **IPT** linker in the presence of methionine, the most sensitive amino acid

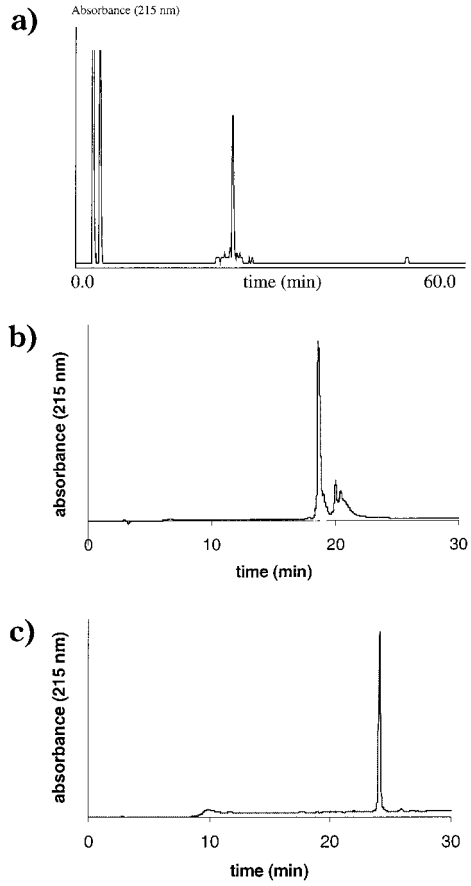


Figure 2. (a) RP-HPLC of crude peptide **6**. (b) Peptide **6** lyophilized without mannitol. (c) Peptide **6** lyophilized with mannitol.

to oxidation by periodate. Peptide **8** was generated by treating the peptidyl resin with sodium periodate in a pH 6.1 citrate buffer/methanol/dimethyl sulfide mixture. The nearly neutral pH and the use of dimethyl sulfide as a cosolvent permitted to minimize the oxidation of methionine into methionine sulfoxide. However, since these mild experimental conditions led to only a partial oxidative cleavage of the tartramide moiety, peptide **8** was isolated with a lower yield (13% yield following RP-HPLC purification, Table, entry 10), together with 2% of Ac-KYM(O)L-NH$(CH_2)_3$NHCOCHO.

Synthesis of the IPT2 and -3 Linkers: Second Approach. To gain flexibility for the synthesis of the linker and to permit the distance between the aldehyde moiety and the peptide or the physical-properties of the spacer to be easily modulated, a second approach was developed where the symmetrical diamine was directly introduced on the solid phase (Scheme 3). The carboxylate **15** was generated by dissolving water and DBU in an excess of **1**. After 1 h at room temperature, the reaction mixture was added to the amino PEGA resin swelled in a minimal volume of DMF. In situ activation of the carboxylate with BOP reagent led to the direct anchoring of the tartrate derivative **15** to the solid support. Reaction of ester resin **16** with an excess of 1,3-diaminopropane or 1,7-diaminoheptane led to the formation of the **IPT2** (0.33 mmol/g) or **IPT3** (0.20 mmol/g) PEGA resins, respectively, which were dried and stored at room temperature as for the **IPT1**-PEGA solid support.

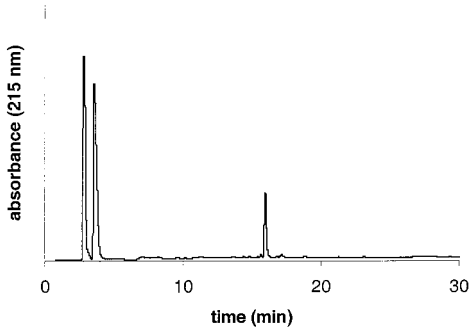
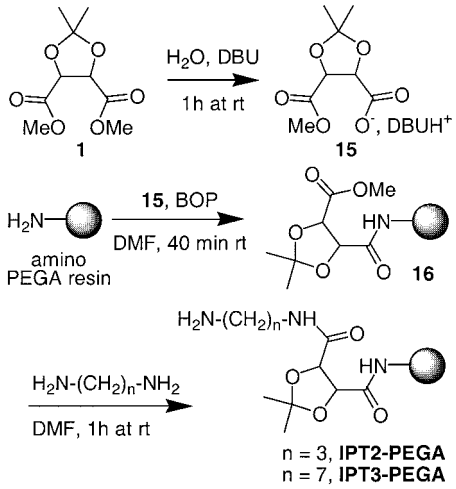


Figure 3. RP-HPLC of crude peptide **9**.

Scheme 3



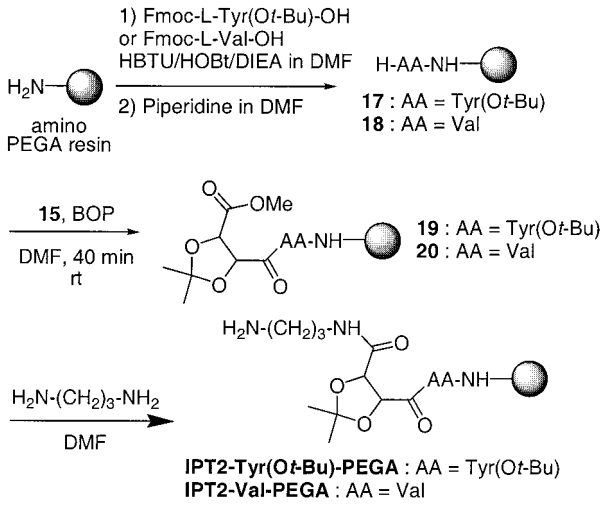
Peptide aldehyde **7** was synthesized using the **IPT2**-PEGA solid support to verify the compatibility of the methodology with Cys containing peptides. The Cys[3] residue was introduced as Fmoc-L-Cys(S-*t*-Bu)-OH. The peptidyl resin was treated with a TFA/water/dimethyl sulfide mixture to remove the Boc, *t*-Bu and acetonide protecting groups. The periodic oxidation was performed in 33% aqueous acetic acid and yielded 52.8% of peptide **7** (Table 1, entry 9), together with 15% of a more hydrophilic compound resulting probably from over oxidation at the disulfide bond.[17]

The sequence Ac-KYVS was assembled on the **IPT3**-PEGA resin. The RP-HPLC profile of the crude peptide **9** resulting from the solid-phase periodic oxidation is depicted in Figure 3 and highlights the efficiency of the process. Peptide **9** was obtained with a 32.9% yield following RP-HPLC purification.

The **IPT2** linker was also assembled on Argogel (**IPT2**-Argogel 0.23 mmol/g) and Novagel (**IPT2**-Novagel 0.33 mmol/g) resins, two poly(ethylene glycol) grafted polystyrene supports. These two **IPT2**-PEG-PS resins were found to be as efficient as the **IPT2**-PEGA one for the synthesis of α-oxo acyl peptides (Table 1, entries 1, 2, and 5). Indeed, peptide **4** was isolated with 38.3 and 38.5% yield using **IPT2**-Argogel and **IPT2**-Novagel resins, respectively.

Optimization of the IPT2 Linker Assembly. As exemplified by peptides **6** and **9** (Figures 2a and 3), the purity of the crude C-terminal peptide α-oxo aldehydes synthesized upon **IPT**-PEGA or PEG-PS solid supports was always found to be high. However, the modest yield obtained for peptide **6** constrasted with the quality of the synthesis, since no loss of material could be ascribed to incomplete couplings or purification difficulties. Acid hydrolysis of the peptidyl resin followed by amino acid analysis revealed that some peptide was still bound to the solid support, which could not be separated from the resin by repeating the periodic oxidation. Thus, the elaboration of the linker was carefully reexamined using HR-MAS NMR as the analytical tool. A Fmoc-L-Tyr(O-*t*-Bu)-OH residue was first coupled to the amino-PEGA resin and used as an internal standard for the evaluation of the NMR peak areas (Scheme 4). (+)-Dimethyl-2,3-*O*-isopropylidene-D-tartrate **1** was anchored to resin **17** as described in Scheme 3. A negative Kaiser test and the HR-MAS NMR spectrum demonstrated the quantitative conversion of **17** into the supported tartramide **19**. Aliquots of resin **19** were finally treated with 1,3-diaminopropane. The duration of the diamine treatment was varied from 5 min to 1 h. The HR-MAS NMR spectra revealed that the displacement of the ester group by the diamine was complete following 20 min of reaction, but a partial loss of the tartramide moiety was also observed. We thus hypothesized that the substitution of Tyr(O-*t*-Bu) by Val, a sterically hindered amino acid, could prevent the premature cleavage of the amide bond linking the tartrate moiety to the solid support. Indeed, treatment of ester resin **20** with 1,3-diaminopropane resulted in a clean transamidation reaction without loss of tartrate. **IPT2**-Val-PEGA resin was dried and stored at room temperature as usual. Synthesis of peptide **4** using this novel solid support led, however, to a disappointing result (21% isolated yield, Table 1, entry 3). A few days later, synthesis of peptide **6** starting from the same resin failed. These results were ascribed to a rapid decomposition of the linker upon storage of the solid support in the dry form. To check this hypothesis, synthesis of peptide **4** was performed again, but starting from a Fmoc-Ser(*O*-*t*-Bu)-**IPT2**-Val-PEGA resin, which was obtained by coupling Fmoc-L-Ser(O-*t*-Bu)-OH immediately following the diamine treatment. In this later case, peptide **4** was isolated with a 58% yield following RP-HPLC purification (Table 1, entry 4). The superiority of this new procedure was verified with longer peptides.

Scheme 4



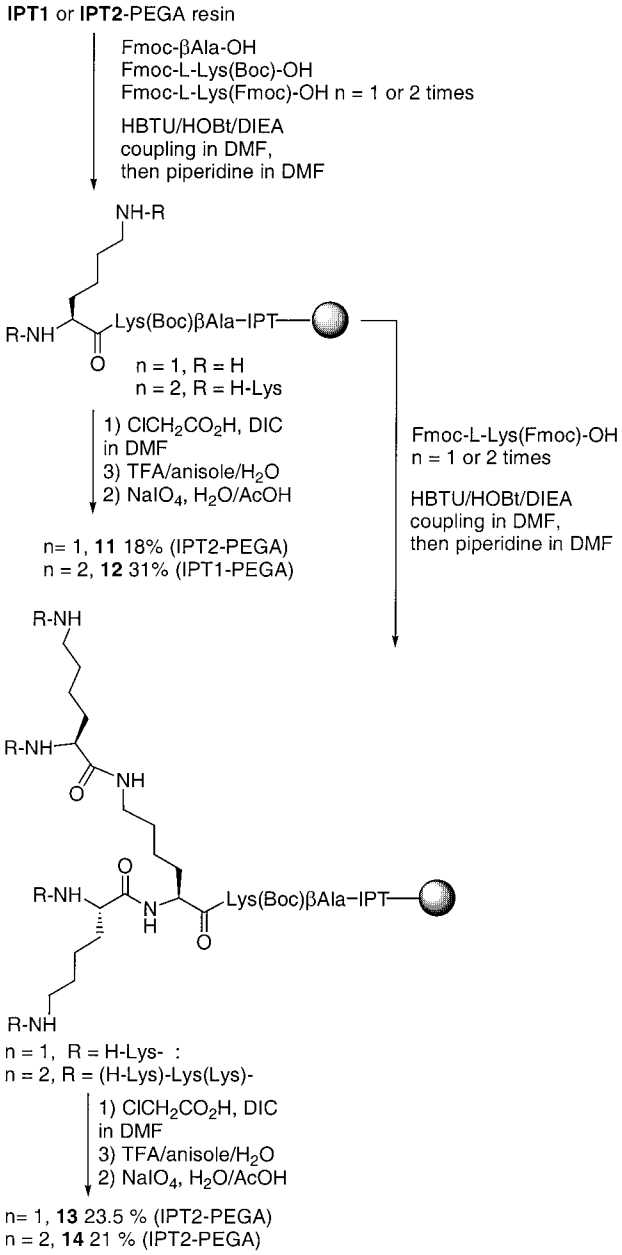
(17) For the thiosulfinate derived from peptide **7**, $[M + H]^+$ calcd 803.01 found 803.3. The oxidation of the disulfide functional group has been extensively studied because of its important role in metabolism. *Organic Sulfur Chemistry: Structure and Mechanism*; Oae, S., Ed.; CRC Press: Boca Raton, 1991; vol. 1, pp 213−216.

For example, decapeptide **10** was obtained with a 52% isolated yield following RP-HPLC purification (Table 1, entry 16). Finally, resynthesis of peptide **6** was undertaken and led to a much better isolated yield (14.6%, Table 1, compare entries 7 and 8). As described before, mannitol was added to the RP-HPLC fractions to avoid the aggregation of the peptide.

Synthesis of Lysyl-Dendrimers 11−14. Bifunctional MAP core matrixes **11**−**14** (Figure 1), functionalized at their surface by chloroacetyl moieties and at the C-terminus by an α-oxo aldehyde group, were described by Grandjean et al. as useful scaffolds for the convergent synthesis of high molecular constructs using "one-pot" double orthogonal ligations.[18] In particular, antigen-bearing lysine based cluster mannosides were assembled by a thioether-hydrazone double-ligation strategy. Lysyl-dendrimers **11**−**14** were synthesized before the optimization of the linker assembly, so that better yields should be obtained using **IPT2**-Val-PEGA resin. Dendrimer **12** was elaborated upon **IPT1**-PEGA resin, whereas structures **11**, **13**, and **14** were obtained starting from the **IPT2**-PEGA solid support. As described in Scheme 5, Fmoc-*β*Ala-OH and Fmoc-L-Lys(Boc)-OH were coupled, respectively, to the solid support using HBTU/HOBt/DIEA activation in NMP. Then, depending on the dendrimer generation, coupling of Fmoc-L-Lys(Fmoc)-OH and deprotection of the a and e amino groups with piperidine was performed one to four times. The peripheral amino groups were then reacted with chloroacetic acid using DIC activation. Removal of the Boc and acetonide protecting groups was performed in a TFA/anisole/water mixture. Lysyl-dendrimers **11**−**13** were generated as usual following treatment of the corresponding deprotected peptidyl resins with sodium periodate in 33% aqueous acetic acid. Compounds **11**−**13** were then easily extracted from the resin by washing with water (Table 1, entries 12−14). However, these experimental conditions failed for dendrimer **14**. MAP dendrimeric cores of the forth generation are known to be hydrophobic structures. Thus, in a second experiment, an organic cosolvent (2-methylpropan-2-ol) was used for the solid-phase periodic oxidation. Dendrimer **14** was successfully extracted at 50 °C with DMSO (21%, Table 1, entry 15).

Chemical Ligation Studies. Peptide **21** was chosen as a test compound for the hydrazone ligation with α-oxo acyl peptide **5**. Indeed, peptides derivatized by a hydrazino acetyl moiety were found to react rapidly with α-oxo aldehydes, and to lead to stable hydrazones.[18] The introduction of a hydrazino acetyl moiety on a peptide can be performed by solid-phase N-electrophilic amination of a glycine residue.[19] Alternately, Grandjean and co-workers have synthesized an N-terminal hydrazino acetyl peptide by reaction of a bromoacetyl group with $BocNHNH_2$ on the solid phase.[18] Finally, Bonnet et al. have recently described the derivatization of the N-terminus of an otherwise protected peptidyl resin by

Scheme 5



$(Boc)_2NN(Boc)CH_2CO_2H$ using BOP activation.[20] The later methodology was used for the synthesis of peptide **21** as described in Scheme 6. Peptides **5** and **21** were reacted 48 h at pH 6.0 in a citrate−phosphate buffer (Scheme 7). Hydrazone **22** was isolated with a 73.6% yield following RP-HPLC purification at pH 2.

Thiazolidine ligation was examined by reacting α-oxo acyl peptide **5** with peptide **23** at pH 5.1 in a citrate−phosphate/NMP mixture in the presence of tris(2-carboxyethyl)phosphine hydrochloride (Scheme 8). After 20 h at 37 °C, the thiazolidine **24** was isolated with a 53.8% yield following RP-HPLC purification. Finally, condensation of peptide **5** with N-terminal aminooxyacetyl peptide **25** was performed at pH 4.8 at room temperature. Oxime **26** was isolated in a 78.1% yield following RP-HPLC purification (Scheme 9).

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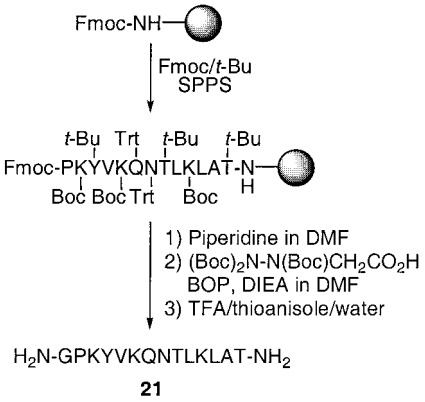
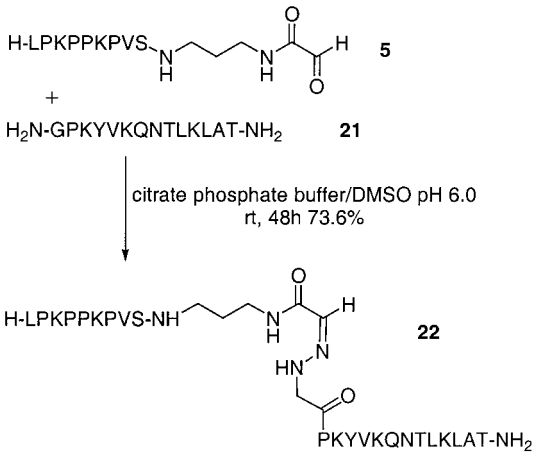
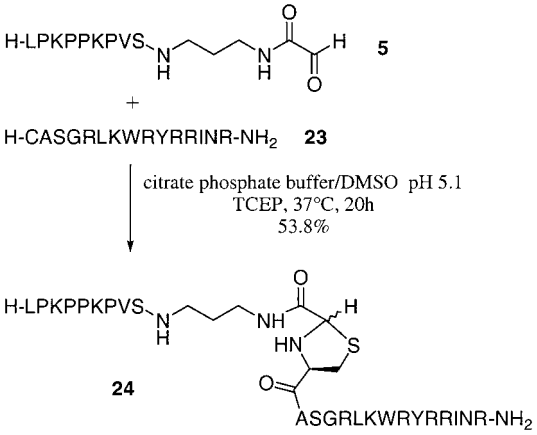
Scheme 6



Scheme 7



Scheme 8

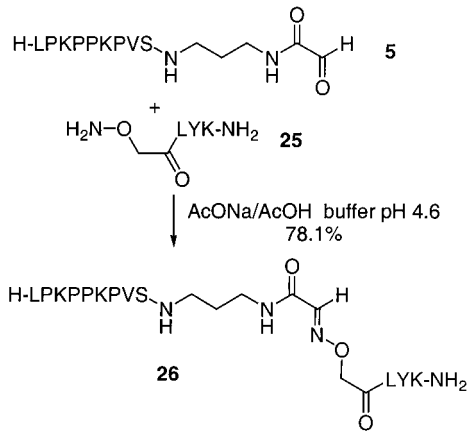


These examples demonstrate the utility of the C-terminal α-oxo peptide aldehydes synthesized in this study for the hydrazone, oxime, and thiazolidine chemical ligations.

Conclusion

We have described the synthesis of a novel tartaric acid-based linker, which allows the synthesis of C-terminal peptide α-oxo aldehydes. The linker was rapidly elaborated on the solid phase starting from (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate and a symmetrical diamine. The peptide was fully deprotected on the resin and separated from the solid support with sodium periodate in various solvent mixtures depending on the sequence and the physicochemical properties of the compound. A C-terminal peptide α-oxo-aldehyde synthesized in this study was reacted with a *N*-alkyl hydrazine, a *β*-aminothiol or a *O*-alkyl hydroxylamine to give a hydrazone, a thiazolidine, or an oxime, respectively. The **IPT** linker should be useful for the automated synthesis of glyoxylic acid derivatives.

Scheme 9



Experimental Section

Analytical and semipreparative RP-HPLC separations were performed on a Beckman system Gold, on Shimadzu LC-10A, LC-9A, or LC-4A systems. Solvent system A: 0.05% TFA in water; solvent system B: 0.05% TFA in acetonitrile/water: 4/1, solvent system C: 0.05% TFA in 2-propanol/water: 2/3. Compounds were verified for homogeneity by analytical Capillary Zone Electrophoresis in a 75 μm × 500 mm fused silica capillary, with a 28 mA current and a 30 kV field in an Applied Biosystems Model 270A-HT system. Separations were performed using a 100 mM sodium borate migration buffer at pH 9.2 and 50 °C or a 20 mM citrate buffer at pH 2.47 and 40 °C.

ESI-MS spectra were recorded on a Micromass Quatro II electrospray mass spectrometer. TOF-PDMS spectra were recorded on a TOF-PDMS Bio-ion 20 plasma desorption mass spectrometer. MALDI-TOF spectra were recorded on a Finnigan Vision 2000 MALDI mass spectrometer. NMR spectra were acquired on a Bruker DRX 300 at a temperature of 298 K. IR spectra were acquired on a Perkin-Elmer Spectrum 1000 FT-IR spectrometer.

Synthesis of 2. A 2 mL (9.16 mmol) portion of (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate (ACROS) was added in 45 min to 10 mL (120 mmol) of 1,3-diaminopropane. The mixture was stirred during 5 h 30 min at room temperature. The excess of diamine was removed under reduced pressure to give a hygroscopic oil that was used without further purification. ^{1}H 300 MHz NMR (DMSO-d_6, TMS) δ: 8.19 (t, 2H, J = 5.5 Hz, CONH), 4.46 (s, 2H, COCH), 3.15 (m, 4H, CH_2NHCO), 2.50 (m, 4H, CH_2NH$_2$), 1.48 (q, 4H, J = 6.6 Hz, NHCH$_2$CH_2), 1.39 (s, 6H, (CH$_3$)$_2$C). ^{13}C 75 MHz NMR (DMSO-d_6, TMS) δ: 171.5, 111.5, 77.6, 39.0, 36.4, 32.6, 26.2. IR (neat, cm^{-1}): 3356.8, 2940.3, 1660.7, 1537.6, 1084.6. TOF-PDMS: [M + H]$^+$ calcd 303.4 found 302.3.

IPT1-PEGA Resin. A 0.4 mmol portion of amino PEGA resin (0.4 mmol/g, Novabiochem) was swelled in a minimal volume of DMF (~9.6 mL). A 400.3 mg (4.0 mmol) portion of succinic anhydride and 697 μL (4.0 mmol) of DIEA dissolved in 2 mL of DMF were added to the beads. The coupling was performed twice (30 min and 1 h). The resin was washed with DMF (2 × 2 min), CH_2Cl_2 (2 × 2 min), and NMP (2 × 2 min).

A 1209 mg (4.0 mmol) portion of **2** dissolved in 1 mL of NMP was added to the succinyl-amino PEGA resin swelled in a minimal volume of NMP. A 265.4 mg (0.6 mmol) portion of BOP reagent was then added in one portion, and the resin was shaken during 1 h. The resin was washed with DMF (4 ×

2 min), CH_2Cl_2 (2 × 2 min), and Et_2O (2 × 2 min) and dried in vacuo. The charge of the resin (0.20 mmol/g) was determined spectrophotometrically by analysis of the dibenzofulvene−piperidine adduct following derivatization of an aliquot with Fmoc-Gly-OH.

IPT2- or 3-Linkers on PEGA, Argogel, or Novagel Resins or H-Val-PEGA Resins. A 7.2 μL (0.4 mmol) portion of water was dissolved in 759 μL of (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate at room temperature. A 59.8 μL (0.4 mmol) portion of DBU was then added, and the mixture was stirred during 1 h. A 0.1 mmol portion of PEGA (0.4 mmol/g), Argogel (0.41 mmol/g), Novagel (0.76 mmol/g), or H-Val-PEGA resin was washed with DIEA 5% in CH_2Cl_2 and DMF, respectively. The above mixture and 177 mg (0.4 mmol) of BOP reagent dissolved in 1 mL of DMF were added successively to the resin swelled in the minimal volume of DMF. The resin was shaken during 40 min and then washed with DMF (4 × 2 min). For Argogel and Novagel resins, the acylation was repeated with BOP dissolved in 2 mL of DMF followed by acetylation with Ac_2O/DIEA/CH_2Cl_2 10/5/85 by volume during 10 min). A 1 mL portion of diamine 7.7 M in DMF was added to the resin swelled in the minimal volume of DMF. After 1 h, the resin was washed with DMF (5 × 2 min), CH_2Cl_2 (2 × 2 min), and Et_2O (2 × 2 min). **IPT2** or **IPT3**-PEGA Argogel or Novagel resins were dried in vacuo: **IPT2**-PEGA (0.33 mmol/g), **IPT3**-PEGA (0.20 mmol/g), **IPT2**-Argogel (0.23 mmol/g), **IPT2**-Novagel (0.33 mmol/g). **IPT2**-Val-PEGA resin was dried in vacuo (Table 1, entry 3) or acylated immediately (Table 1, entries 4, 8, 16) using the last amino acid of the sequence (10 equiv) activated with HBTU/HOBt/DIEA 10 equiv/10 equiv/30 equiv (45 min in DMF at room temperature). The subtitution level of Fmoc-L-Ser(O-*t*-Bu)-**IPT2**-Val-PEGA resin, used for the synthesis of peptide **4**, was found to be 0.24 mmol/g.

Synthesis of Peptides 4−10 on IPT Resins. Peptides **4**−**10** were elaborated on **IPT** resins (see Table 1) using the Fmoc/*tert*-butyl strategy[21] and HBTU/HOBt/DIEA activation in an Applied Biosystem 430 or 431A peptide synthesizer. Fmoc-protected amino acids were purchased from Propeptide (Vert-Le-Petit, France). For peptides **4**−**9**, side-chain protections were as follows: Arg(Pmc), Asn(Tr), Asp(*t*-Bu), Gln(Tr), Lys(Boc), Ser(*t*-Bu), Thr(*t*-Bu), Tyr(*t*-Bu). Peptide **10** was elaborated using Arg(Pbf). Syntheses were performed on a 0.1 mmol scale, and 10 equiv of activated amino acid was used for each coupling step. Single couplings were used for peptides **4** and **7**−**10**. Otherwise, amino acids were coupled twice. The peptidyl-resins were capped with Ac_2O/DIEA/NMP 4.75/2.25/93 by volume containing 7.5 mM HOBt following each coupling.

Deprotection. Peptidyl-resins were deprotected at room temperature with the following mixtures: for peptides **4**, **5**, and **9**, 2 h in 10 mL of TFA/H_2O/anisole 95/2.5/2.5 by volume; for peptide **6**, 3 h in 10 mL of TFA/H_2O/triisopropylsilane 95/2.5/2.5 by volume; for peptide **10**, 3 h in 10 mL of TFA/H_2O/triisopropylsilane 95/2.5/2.5 by volume, for peptide **7**, 2 h in 10 mL of TFA/H_2O/Me_2S 95/2.5/2.5 by volume. For peptide **8**, 2 h in TFA/H_2O/1,2-ethanedithiol/triisopropylsilane 94/2.5/2.5/1 by volume. The resins were washed with CH_2Cl_2 (4 × 2 min) and diethyl ether (2 × 2 min) and then dried under reduced pressure.

Periodic Oxidation. Peptides 4−7 and 9−10. The periodic oxidations were performed by swelling the dry peptidyl-resins (0.1 mmol) with 5 mL of H_2O/acetic acid 2/1 by volume during 15 min. A 128.3 mg (6 equiv) portion of sodium periodate dissolved in 2 mL of H_2O/acetic acid 5/1 was added in one portion, and the suspension was stirred for 2 min. The resins were filtered and washed twice with 6 mL (1 min) of water. For peptides **4**−**9**, the combined solutions were immediately added to 200 μL of ethyleneglycol and injected on a C18 RP-HPLC Hyperprep (15 × 300 mm) column. The products were eluted using a 0.05% TFA water−acetonitrile linear gradient. Peptide **10** was purified by gel filtration on a G10 column using 5% aqueous acetic acid as the mobile phase. Peptides **4**−**5** and **7**−**10** were directly lyophilized.

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Peptide **4**: **IPT1**-PEGA resin 29.0 mg (38%), **IPT2**-Val-PEGA used dry 16.1 mg (21%), **IPT2**-Val-PEGA acylated immediately 38.0 mg (58%), **IPT2**-Argogel used dry 32.7 mg (38.3%), **IPT2**-Novagel used dry 29.4 mg (38.5%), ES-MS $[M + H_2O + H]^+$ $[M + H]^+$ calcd 668.7 650.7, found 668.9 650.8. Amino acid analysis, AA (calcd; found): K(1; 1.0), Y(1; 1.07), V(1; 1.04), S(1; 1.09).

Peptide **5**: 36.8 mg (26%), ES-MS $[M + H_2O + H]^+$ $[M + H]^+$ calcd 1093.3 1075.3, found 1094.2 1076.1. Amino acid analysis, AA (calcd; found): K(2; 1.82), L(1; 1.0), V(1; 0.99), S(1; 0.92), P(4; 4.15).

Peptide **7**: 46.5 mg (52.8%), TOF-PDMS $[M + H_2O + H]^+$ $[M + H]^+$ calcd 803.0 785.0, found 802.7 785.0.

Peptide **9**: 26.0 mg (31.7%), TOF-PDMS $[M + H_2O + H]^+$ $[M + H]^+$ calcd 724.9 706.9, found 725.6 707.9.

Peptide **10**: 114.0 mg (52.0%), MALDI-TOF $[M + H]^+$ calcd 1593.9, found 1593.6.

To the pure fractions of peptide **6** was added (+)-D-mannitol (Sigma) before the lyophilization step. The content of peptide **6** was determined by quantitative amino acid analysis using ninhydrin detection following total acid hydrolysis with 6 N HCl/phenol: 10/1 at 110 °C during 24 h. Peptide **6** was isolated with 6.1% (15.8 mg) and 14.6% (33.1 mg) yield starting from **IPT1**-PEGA resin and **IPT2**-Val-PEGA resins respectively (see Table 1, entries 7 and 8). ES-MS $[M + H]^+$ calcd 2136.6, found 2136.7. Amino acid analysis, AA (calcd; found): Y(1; 0.92), I(3; 2.87), K(4; 3.86), A(1; 0.97), S(1; 0.91), F(1; 0.95), G(1; 0.90), T(1; 0.90).

Peptide **8**. A 270 mg (0.054 mmol) portion of dry peptidyl-resin was suspended in 5.4 mL of sodium citrate buffer (pH 6.0), 5.4 mL of MeOH, and 0.9 mL of Me_2S. A 63.18 mg (0.295 mmol) of $NaIO_4$ dissolved in 2 mL of citrate buffer was added in one portion. The addition of $NaIO_4$ was repeated following 15 min of reaction. The beads were stirred 15 min, filtered, and immediately resuspended in the above sodium citrate/MeOH/Me_2S mixture. The filtrate was immediately added to 0.36 mL of ethyleneglycol. The procedure was repeated 10 times. The filtrates were combined and purified by RP-HPLC on a C18 vydac (15 × 300 mm) column. Peptide **8**: 5.2 mg (13%), ES-MS $[M + H_2O + H]^+$ $[M + H]^+$ calcd 726.9 708.9, found 726.3 708.3. Amino acid analysis, AA (calcd; found): K(1; 0.96), Y(1; 0.84), L(1; 1.04), M(1; 1.08).

Synthesis of Lysinyl Cores 11−14. Lysinyl cores were synthesized on a 0.1 mmol scale on **IPT1** (compound **12**) or **IPT2** (compounds **11**, **13**, and **14**) PEGA resins. The coupling steps were performed using a 10-fold (for the first two coupling steps) or a 4-fold excess of amino acid per amine. Amino acids were coupled using HBTU/HOBt/DIEA activation in NMP. The syntheses were monitored by the TNBS and ninhydrin tests.

Typically, HBTU (4 equiv) was dissolved in NMP and added to a mixture of the amino acid (4 equiv), HOBt (4 equiv), and DIEA (8 equiv) in NMP. After being stirred for 1 min, the mixture was added to the peptidyl resin (1 equiv) swollen in NMP containing DIEA (4 equiv). After 40 min, the resin was filtered and washed with NMP (3 × 2 min) and DCM (3 × 2 min). After each coupling, the resin was acetylated with Ac_2O/DIEA/DCM 10/5/85 by volume for 10 min and washed with CH_2Cl_2 (3 × 1 min). Cleavage of the Fmoc protecting groups was achieved by treatment with 20% piperidine in NMP (1 × 2 min, 1 × 20 min), followed by washings with NMP (2 × 2 min) and DCM (2 × 1 min). Fmoc-β-Ala-OH and Fmoc-L-Lys(Boc)-OH were coupled to the solid support. Then, Fmoc-L-Lys(Fmoc)-OH was coupled one to four times depending on the dendrimer generation. At the end of the dendrimer assembly, the peptidyl resin was deprotected, acylated with $ClCH_2$-COOH/DCC 4 equiv/4 equiv in DMF, washed with DMF (3 × 1 min), CH_2Cl_2 (3 × 2 min), and Et_2O (2 × 1 min), and dried in vacuo. The protecting groups were removed with 10 mL of TFA/H_2O/anisole 95/2.5/2.5 (10 mL) for 2 h at room temperature. The peptidyl resins were then washed with DCM (5 × 1 min), Et_2O (2 × 1 min) and dried in vacuo.

Periodic Oxidation, Dendrimers 11−13. $NaIO_4$ (128.3 mg, 6 equiv) dissolved in 2 mL of 33% aqueous AcOH/H_2O 1/1 was added to the peptidyl resin swollen in 33% aqueous AcOH. The mixture was stirred 5 min at room temperature and then

filtered into 200 μL of ethylene glycol. The resin was washed with H_2O (2 × 6 mL) and the combined filtrates were purified by RP-HPLC, flow rate 3 mL/min, gradient 0−15% B in 25 min for **11**, 0−50% B in 120 min for **12**, 0−10% in 10 min then 10−30% in 40 min then isocratic for **13**. Compounds **11**−**13** were obtained as white powders following lyophilization. **11**: 13 mg (18%) ES-MS $[M + H_2O + H]^+$ $[M + H]^+$ calcd (monoisotopic) 628.3 610.3, found 628.0 610.0. **12**: 28 mg (31%) ES-MS $[M + H_2O + H]^+$ $[M + H]^+$ calcd (monoisotopic) 1036.5 1018.5, found 1036.4 1018.3. **13**: 45 mg (23.5%) ES-MS $[M + H_2O + H]^+$, $[M + H]^+$ calcd 1857.5, 1839.5, found 1855.5, 1838.3.

Periodic Oxidation, Dendrimer 14. An aliquot of the peptidyl resin (111 mg, 0.013 mmol), swollen in 400 μL of 2-methylpropan-2-ol was treated with $NaIO_4$ (17 mg, 6 equiv) and dissolved in 800 μL of 33% aqueous AcOH/H_2O 1/1. The resin was shaken for 15 min, filtered, and washed with 1 mL a mixture of 2-methylpropan-2-ol/AcOH/H_2O 1/1/1 by volume. The lysinyl core was extracted following two washings with DMSO (2 × 2 mL) at 50 °C for 10 min. The combined extracts were diluted in H_2O and purified by RP-HPLC, flow rate 3 mL/min, gradient 0−30% B in 20 min then 30−45% B in 40 min. **14** 9.9 mg (21%) was obtained as a white powder following lyophilization. This compound contained minor impurities as shown by RP-HPLC and CZE. ES-MS M calcd 3475.7, found 3475, 1738.6 $[M + 2H]^{2+}$, 1159.2 $[M + 3H]^{3+}$, 869.8 $[M + 4H]^{4+}$.

Synthesis of Hydrazinopeptide 21. Peptide **21** was elaborated on a Fmoc-PAL-PEG-PS resin (0.20 mmol, 0.16 mmol/g, Perseptive Biosystems) using the Fmoc/*tert*-butyl strategy and HBTU/HOBt/DIEA activation in an Pioneer peptide synthesizer. After peptide elongation, the α-amino group was reacted with $(Boc)_2NN(Boc)CH_2CO_2H$ (93.7 mg, 1.2 equiv) using BOP/DIEA 3.6 equiv/1.2 equiv activation. The resin was washed with DMF, CH_2Cl_2, and diethyl ether and dried in vacuo. The peptide was deprotected and cleaved from the solid support with 10 mL of a TFA/thioanisole/H_2O 90/5/5 by volume during 2 h 30 min. The peptide was precipitated in diethyl ether, centrifugated, dissolved in water/acetic acid: 5/1 and lyophilized. Purification of the crude product was performed by RP-HPLC on a C18 Vydac column (15 × 300 mm), flow rate 3 mL/min, gradient 0−50% C in 90 min, detection at 235 nm, 50 °C. 21 (44.1 mg, 14%) was obtained as a white powder following lyophilization. ES-MS $[M + H]^+$ calcd 1575.9, found 1575.3.

Chemical Ligations. Hydrazone Chemical Ligation, Peptide 22. A 3.5 mg (1.6 mmol) portion of peptide **21** and 6.0 mg (4.2 μmol) of peptide **5** were dissolved in 1.5 mL of citrate−phosphate buffer (pH 6.0) at room temperature. After 48 h, the mixture was purified by RP-HPLC on a C18 Hyperprep column (15 × 300 mm), linear gradient 0−50% B in 50 min, 25 °C. Hydrazone **22** 4.4 mg (73.6%) was obtained as a white powder following lyophilization. ES-MS $[M + H]^+$ calcd 2630.6, found 2630.5.

Thiazolidine Chemical Ligation, Peptide 24. The reaction was performed under N_2 using degassed NMP and citrate−phosphate buffer (pH 5.1). A 6 mg (2.29 μmol) portion of peptide **23** was dissolved in 680 μL of buffer/NMP 1/1 by volume. A 328 μg (1.15 μmol) portion of TCEP dissolved in 48.3 μL of buffer and 6.49 mg (4.58 μmol) of peptide **5** dissolved in 680 μL of buffer/NMP 1/1 by volume were added, respectively. The mixture was stirred for 1 h at room temperature and then kept at 37 °C during 20 h. The mixture was purified by RP-HPLC on a C18 Hyperprep column (15 × 300 mm), flow rate 3 mL/min, gradient 0−30% B in 45 min and 30−50% B in 20 min. **24** (4.8 mg, 53.7% yield) was obtained as a white powder following lyophilization. TOF-PDMS $[M + H]^+$ calcd 2877.5, found 2878.2.

Oxime Chemical Ligation, Peptide 26. A 3.25 mg (5 μmol) portion of peptide **25** and 3.53 mg (2.5 μmol) of peptide **5** were dissolved in 1.4 mL of a 86 mM sodium acetate buffer (pH 4.6) at room temperature. After 48 h, the mixture was purified by RP-HPLC on a C18 Hyperprep column (15 × 300 mm), 3 mL/min, linear gradient 0−50% B in 50 min, 25 °C. Oxime **25** (4.0 mg, 78%) was obtained as a white powder following lyophilization. ES-MS $[M + H]^+$ calcd 1554.7, found 1556.1.

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Supporting Information Available: RP-HPLC, mass spectra, and amino acid analysis of peptides **4**−**10** and **21**. RP-HPLC, capillary zone electrophoresis, and mass spectra of peptides **11**−**14**. RP-HPLC and mass spectra of peptides **22**, **24**, and **26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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