JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

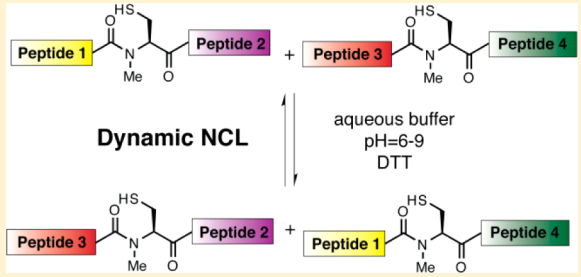
Reversible Native Chemical Ligation: A Facile Access to Dynamic Covalent Peptides

Yves Ruff, Valentina Garavini, and Nicolas Giuseppone*

SAMS Research Group, University of Strasbourg, Institut Charles Sadron, CNRS, 23 rue du Loess, BP 84047, 67034 Strasbourg Cedex 2, France

Supporting Information

ABSTRACT: The broad interest of using reversible covalent bonds in chemistry, in particular at its interfaces with biology and materials science, has been recently established through numerous examples in the literature. However, the challenging exchange of peptide fragments using a dynamic covalent peptide bond has not yet been achieved without enzymatic catalysis because of its high thermodynamic stability. Here we show that peptide fragments can be exchanged by a chemoselective and reversible native chemical ligation (NCL) which can take place at *N*-(methyl)-cysteine residues. This very mild reaction is efficient in aqueous solution, is buffered at physiological pH in the presence of dithiothreitol (DTT), and shows typical half-times of equilibration in the 10 h range.



INTRODUCTION

With a hydrolysis half-time of 7 years in neutral water,[1] the covalent peptide bond is practically inert under physiological conditions. Nevertheless, in living systems, the fast and selective modulation of protein expression and degradation in response to environmental changes requires to continuously form (e.g., ribosome) and break (e.g., proteases) peptide bonds. Typically, the average half-life time of a protein in cells ranges from 1 h to 1 day.[2,3] Most proteins and peptides are thus highly dynamic in their constitution, with this property being instrumental in their biological functions. Synthetic chemists have recently taken advantage of a number of reversible covalent bonds to implement them from drug discovery to material science, opening the so-called field of "dynamic covalent chemistry".[4−9] However, and although very attractive, the use of the peptide bond in dynamic covalent systems is very challenging because of its intrinsic stability. In a couple of examples, chemists succeeded in scrambling pentapeptide inhibitors[10] and in sorting self-assembled dipeptide-based gelators under thermodynamic control[11] in the presence of a protease (thermolysin). Sortase was also shown to form and break peptide bonds, but this particular enzyme is only able to catalyze the ligation of a single specific sequence.[12] Hence, by looking from a more general point of view, the scope of proteases to exchange peptide fragments in synthetic systems has strong restraints due to their limited stability and high substrate specificity; this is why chemists have envisioned alternative approaches. For instance, Gellman and co-workers[13,14] and Ashkenasy et al.[15] have used thioester linkages as dynamic surrogates of peptide bonds in helical and β-sheets secondary structures. Complementary attempts to exchange nonpeptidic secondary amides by zirconium-catalyzed metathesis reactions were performed by Gellman et al., but with restriction to nonaqueous systems.[16] Therefore, and despite its manifest potential toward several domains of research, the design of an abiotic (i.e., enzyme free) synthetic approach to perform dynamic covalent formation, disruption, and exchange of peptide bonds on a practical time scale and in mild conditions is unprecedented. Here we describe a new methodology based on native chemical ligation (NCL) to prepare dynamic covalent peptides at neutral pH with fast exchange rates.

NCL is one of the most popular tools for the synthesis of large peptides and small proteins.[17−20] The power of this nonreversible reaction resides in the straightforward formation of a native peptide bond from a C-terminal thioester and a N-terminal cysteine under physiological conditions. Interestingly, and although NCL is not a chemical reaction involved in any biochemical process, a mechanism similar to a reverse NCL reaction (retro-NCL) is found in the self-splicing of inteins.[21−23] It is proposed that, in the folded intein, the scissile bond at the cysteine residue is preferentially in a *cis* or distorted conformation favoring the intramolecular nucleophilic attack of the cysteine thiol[21,22] (N → S acyl shift, Figure 1a) with an equilibrium constant 4000 times larger than that of the corresponding *trans* conformation.[23] In synthetic chemistry, peptide thioesters have been prepared by fragmentation of C-terminated cysteine peptides using the retro-NCL, but under harsh acidic conditions and in the presence of a large excess of an aliphatic thiol.[24,25] This methodology is also limited to C-terminal cysteine peptides, and furthermore, these conditions are not compatible with the "forward" NCL that requires neutral pH and room temperature.[17] Very recently, peptides containing a C-terminal bis(2-sulfanylethyl)amino group were

Received: December 24, 2013
Published: April 9, 2014

   dx.doi.org/10.1021/ja4129845 | *J. Am. Chem. Soc.* 2014, 136, 6333−6339

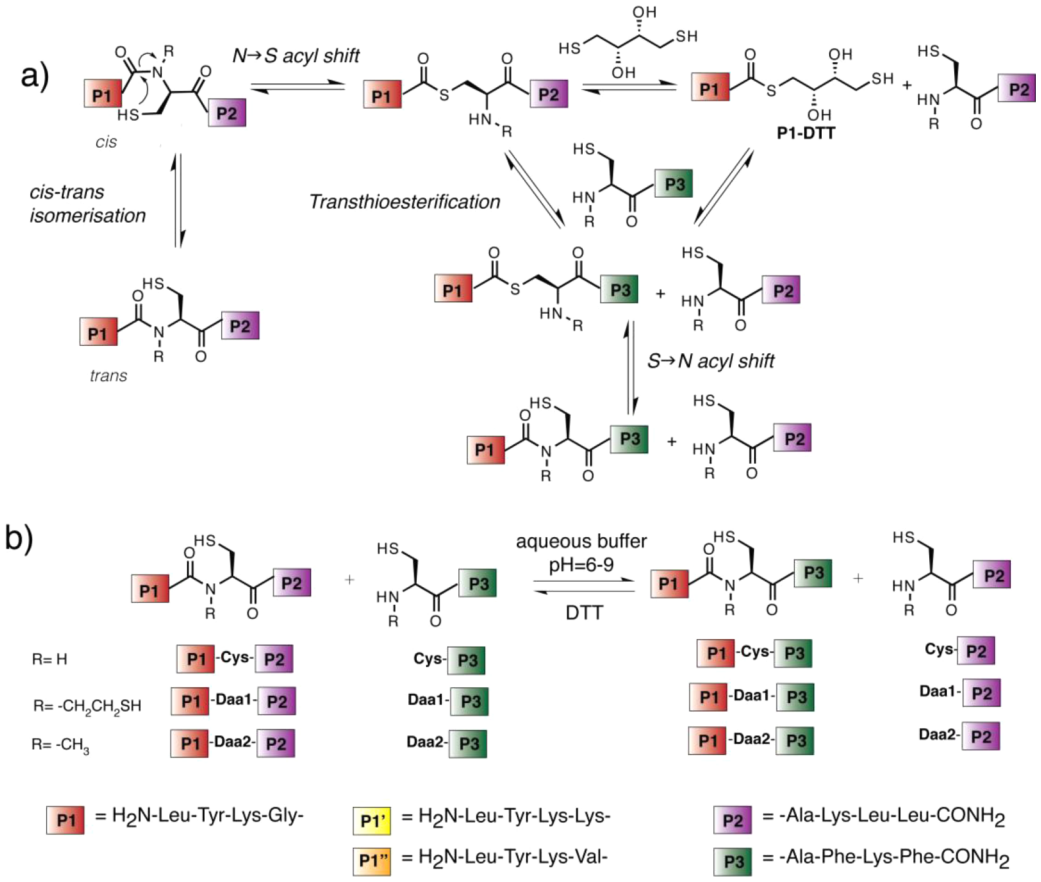


Figure 1. (a) Coupled equilibria describing a fully dynamic NCL which involves sequences of *cis-trans* isomerizations, N → S acyl shifts, transthioesterifications, and S → N acyl shifts. Intermediate P1-DTT which is produced by the presence of dithiothreitol additive in the present study is represented for the sake of clarity. See also Scheme S1 in the Supporting Information for the detailed mechanism involving the particular case of Daa1 which can involve two kinds of N → S acyl shifts. (b) Chemical structures of the peptide fragments (P1, P1′, P1″, P2, and P3) used in this study and schematic representation of a dynamic NCL based on the use of cysteine (Cys), *N*-(2-thioethyl)-cysteine (Daa1), and *N*-(methyl)-cysteine (Daa2). All peptides have a C-terminal carboxamide.

shown independently by the groups of Melnyk and co-workers and Liu et al.[26−28] to undergo N → S acyl shift under neutral (pH 7 and 37 °C) to mildly acidic (pH 4 to 6) aqueous conditions. The unprecedented reactivity of this tertiary amide was explained by the fact that in bis(2-sulfanylethyl)amino derivatives, the need for *cis-trans* isomerization prior to the N → S acyl shift is obviated as there is always one thiol group correctly positioned for the intramolecular thiolysis to occur. By modifying and extending further these lines of investigations, we describe hereafter a fully dynamic covalent version of the NCL reaction.

RESULTS AND DISCUSSION

As a starting point of investigation, we decided to replace both internal and N-terminal cysteine residues by a novel non-natural "dynamic amino acid" Daa1 (Figure 1b, R = CH_2CH_2SH). In this configuration, the new exchange products after transthioesterification and S → N acyl shift would in turn incorporate an internal N-(2-thioethyl)-cysteine and should undergo itself a N → S acyl shift (either by the cysteine or the thioethyl moiety; see all the possible intermediates in Scheme S1 of the Supporting Information (SI)). One can expect such a system to be controlled by thermodynamics and thus suitable for the design of dynamic covalent peptides. We synthesized short model peptides P1−P3 with random sequences using conventional or microwave assisted solid phase synthesis in the Fmoc strategy (detailed synthetic protocols and full characterizations of all peptides are described in the SI).

The incorporation of the new 2-thioethyl unit on the N-terminal free amines of peptides immobilized on solid support was adapted from synthetic routes described for bis(2-sulfanylethyl)amino derivatives.[28] For practical reasons, peptides P1-Daa1-P2 (Leu-Tyr-Lys-Gly-Daa1-Ala-Lys-Leu-Leu-NH_2) and Daa1-P3 (Daa1-Ala-Phe-Lys-Phe-NH_2) were isolated in their oxidized form containing an intramolecular disulfide bond. These model peptides were then used to study the dynamic of the NCL by following the exchange reaction of peptides P1-Daa1-P2 with Daa1-P3 at pH = 6 in a phosphate buffer and in the presence of 0.38% w/v of dithiothreitol DTT (an additive which is added to avoid disulfide formation from thiols in the reaction mixture and which is the reference reducing agent commonly used for protein assays or even directly in cells).[29−31]

The dynamic covalent character of the NCL was unambiguously established by the appearance of peptides Daa1-P2 and P1-Daa1-P3 (which were also prepared independently as references; see the SI). Typical reversed phase chromatograms and kinetic plots recorded during the course of the reaction from an ultra performance liquid chromatography coupled to UV spectroscopy and mass spectrometry units (Waters UPLC-MS) are presented in Figure 2. Importantly, the symmetric reaction consisting in the

dx.doi.org/10.1021/ja4129845 | J. Am. Chem. Soc. 2014, 136, 6333−6339

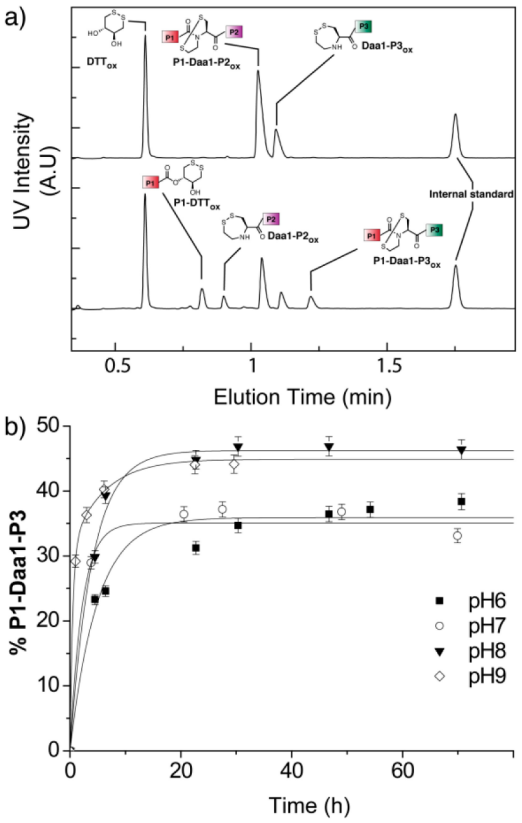


Figure 2. (a) Reversed phase chromatograms (UPLC) recorded with UV and MS detection of the exchange reaction between P1-Daa1-P2 and Daa1-P3 at pH = 6 and at t = 0 (top) and t = 46 h (bottom). As a consequence of the dilution of the aliquot with a H_2O_2 solution, all peptides were observed in their oxidized disulfide form. Upon oxidation, thioester P1-DTT forms P1-DTT$_{ox}$ with intramolecular disulfide bond from its ester form according to UPLC-MS data (see SI Figure S3). (b) Percent of exchanged product P1-Daa1-P3 (i.e., concentration ratio of peptides [P1-Daa1-P3]/([P1-Daa1-P2]+[P1-Daa1-P3])), as a function of time, and starting from solutions of P1-Daa1-P2 and Daa1-P3 at a concentration of 2.5 mM and at pH 6, 7, 8, and 9 (20 °C), in a phosphate buffer (200 mM and 0.38% w/v of DTT). The trend lines are used simply to guide the reader's eye. Concentrations and error bars were calculated from calibration curves and uncertainties on experimental volumes (see the SI).

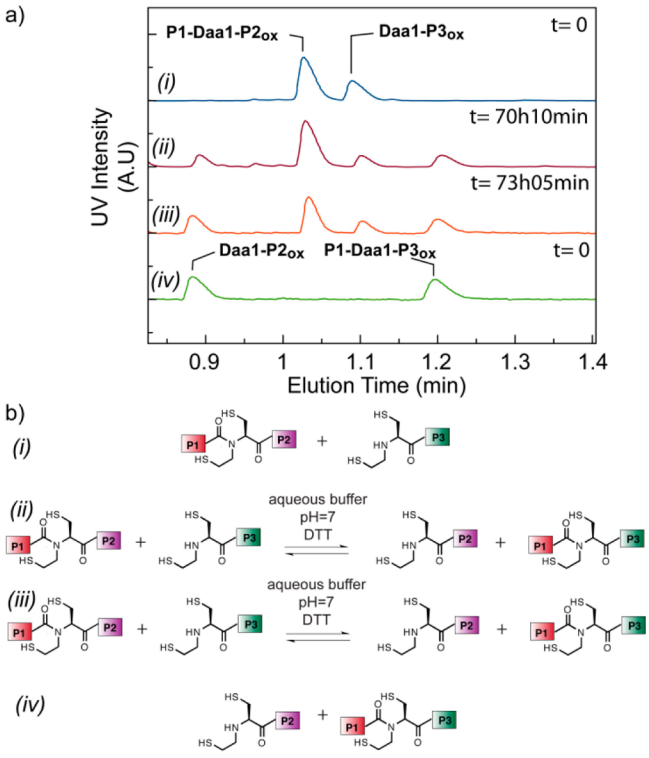


Figure 3. (a) Reversed phase chromatograms (UPLC) recorded with UV and MS detection (at t = 0 (i, iv) and t = equilibrium (ii, iii)) for both the forward exchange reaction (starting from P1-Daa1-P2 and Daa1-P3) and backward exchange reaction (starting from P1-Daa1-P3 and Daa1-P2) described in (b). Reaction performed in aqueous phosphate buffer at pH = 7.

dynamic exchange starting from P1-Daa1-P3 and Daa1-P2 led to the same peptide distribution, proving the thermodynamic control of the system (Figure 3).

This reactivity is in contrast with the control experiment performed on peptides P1-Cys-P2 and Cys-P3. At pH7, and after 4 weeks, we could not detect any trace of the exchange products P1-Cys-P3 and Cys-P2 (see SI Figure S1). Experimentally, the crude exchange mixtures were separated on UPLC HSS-T3 columns and quantitatively analyzed only after the full oxidation of all peptides in their corresponding intramolecular disulfides. The method consists in diluting the exchange reaction with a H_2O_2 solution containing an internal UV standard (3,5-dimethoxybenzoic acid) to account for potential errors during the sample preparation and injection process (see SI text and Figure S2). We then covered a pH range going from mildly acidic to mildly basic (Figure 2b). The dynamic recombination of P1-Daa1-P2 with Daa1-P3 in water leads to the rapid formation of the exchange products P1-Daa1-P3 and Daa1-P2 at all tested pH, with initial rates (V_0) of 6.94 $\times 10^{-8}$, 8.33 $\times 10^{-8}$, 1.11 $\times 10^{-7}$, and 1.53 $\times 10^{-7}$ M·s^{-1}; and half-times of equilibration ($t_{1/2}$) of 5.5, 2, 4, and 1.5 h, at room temperature and at pH 6, 7, 8, and 9 respectively.

This rate dependency on pH can be explained according to the literature on both consecutive individual steps that are required to yield the exchange product P1-Daa1-P3. Indeed, the rearrangement of amide-based bis(2-sulfanylethyl)amino units into their thioester form is favored by lowering the pH,[26,27] suggesting that, in addition to the increase of the cis amide bond population, inhibition of the S → N acyl shift by protonation of the amide could shift the equilibrium toward thioester formation. With the particular case of our exchanging unit, the formation of two distinct thioesters from P1-Daa1-P2 was confirmed at low pH (see SI Figure S10a). Conversely, the second determining step of the dynamic NCL pathway, namely the transthioesterification, is accelerated by increasing the pH to mildly basic conditions.[32] It is therefore interesting that in this dynamic exchange, the fastest initial rate at pH = 9 suggests the transthioesterification as the rate limiting step. In addition to the expected exchange products, we also observed at lower pHs the presence of P1-DTT resulting from the trans-thioesterification between the thioester generated by the N → S acyl shift of P1-Daa1-P2 and DTT (Figures 1a and 2a). P1-DTT is in equilibrium with all other components of the mixture and is constantly regenerated during the exchange process, which is thus not detrimental to the dynamic of the system. Interestingly, at pH 8, the proportion of P1-DTT at equilibrium is only residual while at pH 9 it is not even detectable (Figure 4).

dx.doi.org/10.1021/ja4129845 | J. Am. Chem. Soc. 2014, 136, 6333−6339

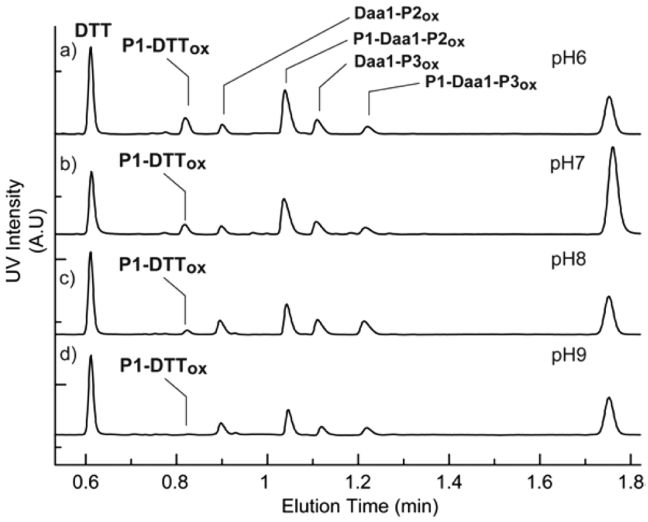


Figure 4. Reversed phase chromatograms (UPLC) recorded with UV and MS detection of exchange reactions between P1-Daa1-P2 and Daa1-P3, leading to P1-Daa1-P3 and Daa1-P2, and showing the P1-DTT thioester intermediates as a function of pH values (from 6 to 9). All chromatograms are given at thermodynamic equilibrium.

However, at this later pH and long time after equilibration (i.e., it can be neglected in the time scale of the exchange), residual hydrolysis of thioester intermediates is observed, leading to small amounts of the C-terminal free carboxylic peptide Leu-Tyr-Lys-Gly (see SI Figure S11). Thus, in order to keep the fastest exchange rate and to avoid long-term hydrolysis, one can consider that the optimal pH value for this dynamic process is close to 7. Finally, as already described in the literature for dynamic covalent libraries involving individual pH-dependent mechanisms, we observe that the equilibrium constant (K) is also strongly affected by the medium, in particular when varying pH (and/or temperature), and leads to differential expressions of the products at thermodynamic equilibrium for each set of conditions.[33,34]

Although leading to the first experimental example of a dynamic NCL mechanism, the *N*-(2-thioethyl)-cysteine incorporated as a reversible junction is not by itself a natural amino acid. Therefore, the potential biocompatibility and bioactivity of the resulting compounds would remain uncertain. We have thus probed which minimal modification of cysteine could lead to a reversible amide junction on a practical time scale while getting even closer to natural peptides. In particular, reasoning that the *cis* conformation could be favored even by an elementary steric hindrance on the nitrogen atom, we have turned our investigations to *N*-(methyl)-cysteine.

Similarly to *N*-(2-thioethyl)-cysteine, *N*-alkyl and in particular *N*-(methyl)-cysteine are indeed known to promote the rearrangement of peptides into thioesters, presumably also by N → S acyl shifts under acidic conditions.[35] For instance, this residue has been used to generate peptide thioesters in situ for native chemical ligation in the synthesis of small proteins.[36] More recently, *N*-(methyl)-cysteine has also been shown to promote the N → S acyl shift in the mechanistic studies of model inteins.[22] Interestingly, *N*-(methyl)-cysteine is a non-ribosomal amino acid that can be found in natural products.[37] *N*-Methylation is also a common post-translational modification taking place in many microbial peptides such as cyclosporins.[38] Furthermore, the incorporation of *N*-methyl amino acids into peptides have been shown to improve proteolytic stability,[39] to increase selectivity in biomolecular recognition processes,[40] to inhibit amyloid[41] and polyglutamine[42] peptide aggregation, and to promote the diffusion of peptides through the blood-brain barrier.[43] Finally, the synthesis of *N*-methyl-peptides is well documented[44] and, in our hands, was found less challenging than for peptides incorporating the *N*-(2-thioethyl)-cysteine residue (see the SI for detailed protocols).

N-(Methyl)-cysteine (Daa2) was incorporated in our model peptides P1-Daa2-P2 and Daa2-P3 which were subsequently equilibrated in solution at various pH and temperatures. The reaction was followed by UPLC-MS (Figure 5a) and, for each

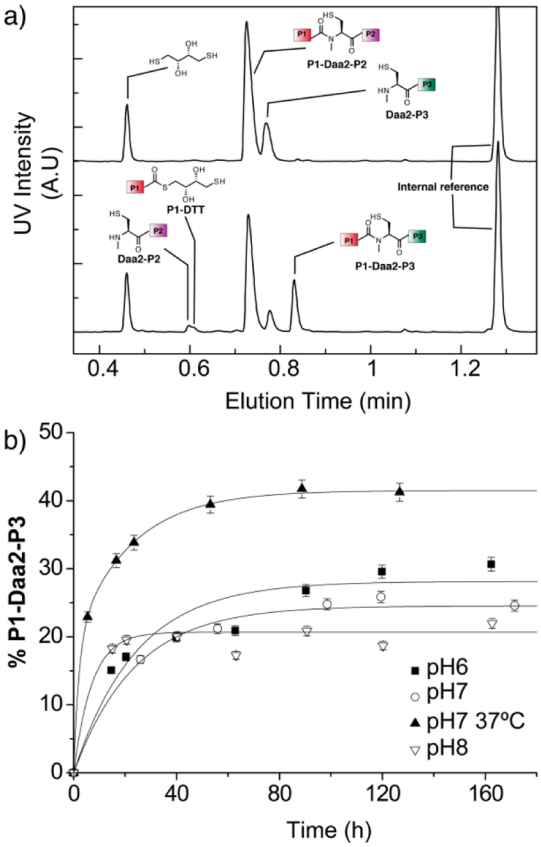


Figure 5. (a) Reversed phase chromatograms (UPLC) recorded with UV and MS detection of the exchange reaction between P1-Daa2-P2 and Daa2-P3 at pH 7 and at t = 0 (top) and t = 98 h (bottom). (b) Percent of exchanged product P1-Daa2-P3 (i.e., concentration ratio of peptides [P1-Daa2-P3]/([P1-Daa2-P2] + [P1-Daa2-P3])), as a function of time, and starting from solutions of P1-Daa2-P2 and Daa2-P3 at a concentration of 2.5 mM and at pH 6, 7, 8 (20 °C), and at pH 7 (37 °C), in a phosphate buffer (200 mM and 0.38% w/v of DTT). The trend lines are used simply to guide the reader's eye. Concentrations and error bars were calculated from calibration curves and uncertainties on experimental volumes (see the SI).

time point, an aliquot of the exchange mixture was diluted with a solution containing tris(2-carboxyethyl)phosphine (TCEP) – for the characterization of the eluted peptides in their reduced thiol forms – and containing an internal UV standard (3,5-dimethoxybenzoic acid). Satisfyingly, the expected exchange products P1-Daa2-P3 and Daa2-P2 were shown to exchange at room temperature for pH 6, 7, and 8, giving initial rates (V_0) of 1.11×10^{-8}, 1.38×10^{-8}, and 4.16×10^{-8} M.s^{-1}; and half-time of equilibration ($t_{1/2}$) of 25, 20, and 6.0 h respectively (Figure

dx.doi.org/10.1021/ja4129845 | J. Am. Chem. Soc. 2014, 136, 6333–6339

5b). The average rates of the reactions in the presence of Daa2 are thus slightly slower than in the presence of Daa1, which is not surprising regarding the reduced effective molarity of the internal monothiol when using N-(methyl)-cysteine instead of bisthiol N-(2-thioethyl)-cysteine. Interestingly, this decrease might also explain the only residual expression of thioester P1-DTT in the case of Daa2, even at pH = 6, leaving the library almost fully displaced toward its condensed peptides while exchanging their fragments at all pHs. Finally, the dynamic of the system was studied at pH 7 and at 37 °C, revealing a set of kinetic parameters ($t_{1/2}$ = 10 h and V_0 = 5.00 × 10^{-8} M·s^{-1}) which ranges in a reasonable time scale to extend dynamic covalent chemistry with peptides in biologically compatible conditions.

To get a better insight in the possible extends of our methodological approach, we then turned to another type of exchange reaction involving Daa2 and which consists in starting from two preformed peptides, both containing only internal dynamic residues (i.e., not involving a terminal dynamic cysteine). For this purpose, a new peptide P1′-Daa2-P2 (Leu-Tyr-Lys-Lys-Daa2-Ala-Lys-Leu-Leu-NH_2) was synthesized and the exchange reaction was performed with P1-Daa2-P3 in a phosphate buffer (pH 7) containing 1.5% w/v of DTT, and at a temperature of 37 °C (Figure 6). Satisfyingly, we unambiguously characterized the clean exchange reaction by UPLC-MS, with the appearance of the two new cross-products P1′-Daa2-P3 and P1-Daa2-P2 and with a half-time of equilibration of 12 h.

Ultimately, in order to probe the limit of this reaction, we have introduced a valine residue (instead of the glycine or lysine ones) in α-position of the N-(methyl)-cysteine, which is among the most reluctant residues for the classical NCL reaction (and prohibitively slow even in the presence of a thiophenol catalyst)[19] (Figure 1b, P1″-Daa2-P2). Interestingly, despite this very unfavorable configuration and although thermodynamic equilibrium was not reached, we were pleased to characterize the exchange products at pH 7 or pH 8 and 37 °C after 3 days (see SI Figure S5). In this particular exchange, no trace of P1″-DTT was detected (conversely to the cases of glycine or lysine residues), indicating a difficult transthioesterifcation step.[19] These combined observations in the presence of valine next to the cysteine strongly reinforces the claim that our methodology is closely related to, and might encompass the broad scope of, the original nonreversible NCL.

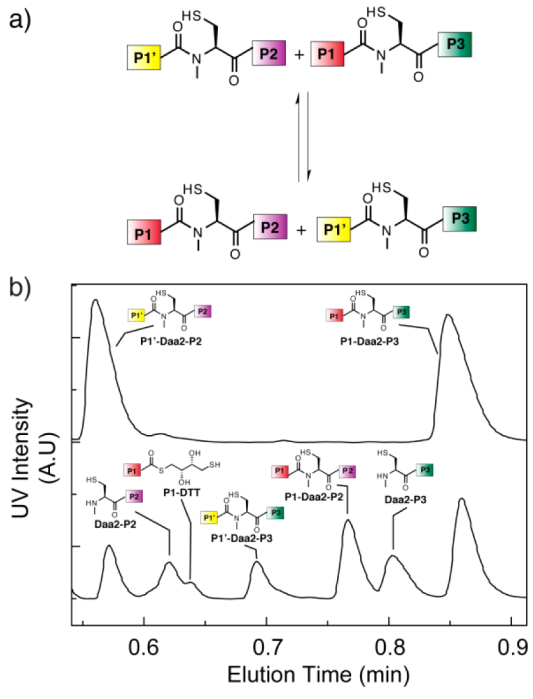


Figure 6. (a) Schematic representation of the exchange reaction performed between two peptides incorporating internal dynamic units P1′-Daa2-P2 and P1-Daa2-P3. (b) Corresponding reversed phase chromatograms (UPLC) recorded with UV and MS detection of the exchange reaction described in (a) at t = 0 (top) and at thermodynamic equilibrium (t = 48 h) (bottom) for an initial concentration of 5.0 mM of each peptide, at pH 7 (37 °C) and in a phosphate buffer (200 mM with 1.5% w/v of DTT).

CONCLUSION

In conclusion, small modifications of a cysteine residue in polypeptides have been used to activate this specific peptide bond for exchange reactions. Our dynamic design of the NCL has been here refined up to the minimal incorporation of a N-(methyl)-cysteine which is biologically relevant. The time scale of the exchange reaction in physiological conditions and in the presence of DTT appears compatible with a broad range of bioassays and beyond.[45] Importantly, this methodology allows for a high degree of control on the location of the reversible junctions in dynamic peptides or proteins as the process is entirely orthogonal to the other peptide bonds. Because the original NCL methodology can be further used to create peptide bonds even without a cysteine residue,[18] as long as thiol groups are introduced by the intermediate of N-alkyl side chains (such as in Daa1), one can envision that the scope of our dynamic version can be very large and encompass other types of amide bonds. Overall, this new methodology for creating dynamic covalent peptides and its potential developments seem of high interest for the finding or the release of bioactive molecules,[5] for the study of complex chemical systems,[46] and for the development of dynamic functional materials.[47,48]

EXPERIMENTAL SECTION

Protocols for Exchange Reactions. *Peptides Incorporating N-(2-Thioethyl)-cysteine.* Phosphate buffers of 0.2 M were prepared at pH 6, 7, 8 and 9: the amount of NaH_2PO_4 required to give a 200 mM solution in 20 mL of water (551 mg) was dissolved in 10 mL of water. The resulting acidic solution was titrated to pH 6, 7, 8, or 9 using 2 M NaOH, and its volume adjusted to 20 mL to give 0.2 M buffer solutions at these pHs. Buffers were degassed using freeze/thaw cycles and placed under argon prior to use.

Daa1-P3 (formate salt) was dissolved in water to give a solution A (5 mM). P1-Daa1-P2 (diformate salt) was dissolved in water to give a solution B (5 mM). A volume of 250 μL of solution A was mixed with 250 μL of solution B and diluted with a solution C of TCEP (10 mM, 7.1 mg in 2.5 mL of water neutralized with 8.4 mg of $NaHCO_3$, 2 eq., 250 μL) to give a solution D. This solution D was immediately frozen and lyophilized. The lyophilized powder of the mixed peptides was diluted under argon with 500 μL of phosphate buffer (pH 6, 7, 8 or 9) containing 25 mM DTT (5.8 mg in 1.5 mL).

Peptides Incorporating N-(Methyl)-cysteine. Phosphate buffers of 0.2 M were prepared as described above at pH 6, 7, and 8. They were degassed and stored under Ar.

Daa2-P3 (formate salt) was dissolved in water to give a solution A (5 mM). P1-Daa2-P2 (diformate salt) was dissolved in water to give a solution B (5 mM). A volume of 250 μL of solution A was mixed with 250 μL of solution B, and this mixture was immediately frozen and lyophilized. The lyophilized powder of the mixed peptides was diluted

dx.doi.org/10.1021/ja4129845 | J. Am. Chem. Soc. 2014, 136, 6333−6339

under argon with 500 μL of phosphate buffer (pH 6, 7 or 8) containing 25 mM DTT (5.8 mg in 1.5 mL).

In order to monitor these exchange reactions, for each time point, an aliquot of the reaction (25 μL) was diluted with 175 μL of a dilute TCEP solution containing an internal UV standard (40 μL 3,5-dimethoxybenzoic acid solution (5 mM in a 0.2 M pH 7 phosphate buffer) and 960 μL of a 10 mM TCEP·HCl solution in water). The resulting solution was filtered and injected in the UPLC-MS apparatus (2 × 2 μL injections).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic protocols and characterization of peptides as well as all details about the mechanistic aspects. This material is available free of charge via the Internet at http://pubs.acs.org."

■ AUTHOR INFORMATION

Corresponding Author

giuseppone@unistra.fr

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research leading to these results has received funding from the European Research Council under the European Community's Seventh Framework Program (FP7/2007-2013)/ERC Starting Grant Agreement No. 257099 (N.G.). We wish to thank the Centre National de la Recherche Scientifique (CNRS), the COST action (CM 1304), the international center for Frontier Research in Chemistry (icFRC), the Laboratory of Excellence for Complex System Chemistry (LabEx CSC), the University of Strasbourg (UdS), and the Institut Universitaire de France (IUF) for financial support. This work was also supported by a Marie Curie Fellowship Career Integration Grant (292281 NaDyPe) (Y.R.), and by a Marie Curie Initial Training Network (PITN-GA-2011-289033 Dynano) (V.G.). We thank Ms. Mélodie Archimbaud for her contribution in peptide purification.

■ REFERENCES

(1) Kahne, D.; Still, W. C. *J. Am. Chem. Soc.* **1988**, *110*, 7529−7534.
(2) Eden, E.; Geva-Zatorsky, N.; Issaeva, I.; Cohen, A.; Dekel, E.; Danon, T.; Cohen, L.; Mayo, A.; Alon, U. *Science* **2011**, *331*, 764−768.
(3) Belle, A.; Tanay, A.; Bitincka, L.; Shamir, R.; O'Shea, E. K. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13004−13009.
(4) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898−852.
(5) Ramström, O.; Lehn, J.-M. *Nat. Rev. Drug Discovery* **2002**, *1*, 26−36.
(6) Moulin, E.; Cormos, G.; Giuseppone, N. *Chem. Soc. Rev.* **2012**, *41*, 1031−1049.
(7) Giuseppone, N. *Acc. Chem. Res.* **2012**, *45*, 2178−2188.
(8) *Constitutional Dynamic Chemistry*; Barboiu, M. Ed.; Topics in Current Chemistry; Springer: Berlin, Heidelberg, 2012; Vol. 322.
(9) Montarnal, D.; Capelot, M.; Tournilhac, F.; Leibler, L. *Science* **2011**, *334*, 965−968.
(10) Swann, P. G.; Casanova, R. A.; Desai, A.; Frauenhoff, M. M.; Urbancic, M.; Slomczynska, U.; Hopfinger, A. J.; Le Breton, G. C.; Venton, D. L. *Biopolymers* **1996**, *40*, 617−625.
(11) Williams, R. J.; Smith, A. M.; Collins, R.; Hodson, N.; Das, A. K.; Ulijn, R. V. *Nat. Nanotechnol.* **2009**, *4*, 19−24.
(12) Williamson, D. J.; Fascione, M. A.; Webb, M. E.; Turnbull, W. B. *Angew. Chem., Int. Ed.* **2012**, *51*, 9377−9380.
(13) Hadley, E. B.; Witek, A. M.; Freire, F.; Peoples, A. J.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2007**, *46*, 7056−7059.
(14) Price, J. L.; Hadley, E. B.; Steinkruger, J. D.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2010**, *49*, 368−371.
(15) Dadon, Z.; Samiappan, M.; Shahar, A.; Zarivach, R.; Ashkenasy, G. *Angew. Chem., Int. Ed.* **2013**, *52*, 9944−9947.
(16) Stephenson, N. A.; Zhu, J.; Gellman, S. H.; Stahl, S. S. *J. Am. Chem. Soc.* **2009**, *131*, 10003−10008.
(17) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776−779.
(18) Marinzi, C.; Bark, S. J.; Offer, J.; Dawson, P. E. *Bioorg. Med. Chem.* **2001**, *9*, 2323−2328.
(19) Johnson, E. C. B.; Kent, S. B. H. *J. Am. Chem. Soc.* **2006**, *128*, 6640−6646.
(20) Raibaut, L.; Ollivier, N.; Melnyk, O. *Chem. Soc. Rev.* **2012**, *41*, 7001−7015.
(21) Volkmann, G.; Mootz, H. *Cell. Mol. Life Sci.* **2013**, *70*, 1185−1206.
(22) Binschik, J.; Mootz, H. D. *Angew. Chem., Int. Ed.* **2013**, *52*, 4260−4264.
(23) Paulus, H. *Annu. Rev. Biochem.* **2000**, *69*, 447−496.
(24) Kang, J.; Richardson, J. P.; Macmillan, D. *Chem. Commun.* **2009**, 407−409.
(25) Macmillan, D.; Adams, A.; Premdjee, B. *Isr. J. Chem.* **2011**, *51*, 885−899.
(26) Ollivier, N.; Dheur, J.; Mhidia, R.; Blanpain, A.; Melnyk, O. *Org. Lett.* **2010**, *12*, 5238−5241.
(27) Dheur, J.; Ollivier, N.; Vallin, A. L.; Melnyk, O. *J. Org. Chem.* **2011**, *76*, 3194−3202.
(28) Hou, W.; Zhang, X.; Li, F.; Liu, C.-F. *Org. Lett.* **2011**, *13*, 386−389.
(29) Cleland, W. W. *Biochemistry* **1964**, *3*, 480−482.
(30) Jocelyn, P. C. *Methods Enzymol.* **1987**, *143*, 246−256.
(31) Tatu, U.; Braakman, I.; Helenius, A. *EMBO J.* **1993**, *5*, 2151−2157.
(32) Bracher, P.; Snyder, P.; Bohall, B.; Whitesides, G. *Origins Life Evol. Biospheres* **2011**, *41*, 399−412.
(33) Giuseppone, N.; Lehn, J.-M. *Chem.—Eur. J.* **2006**, *12*, 1715−1722.
(34) Tauk, L.; Schröder, A.; Decher, G.; Giuseppone, N. *Nat. Chem.* **2009**, *1*, 649−656.
(35) Hojo, H.; Onuma, Y.; Akimoto, Y.; Nakahara, Y.; Nakahara, Y. *Tetrahedron Lett.* **2007**, *48*, 25−28.
(36) Erlich, L. A.; Kumar, K. S. A.; Haj-Yahya, M.; Dawson, P. E.; Brik, A. *Org. Biomol. Chem.* **2010**, *8*, 2392−2396.
(37) Pérez Baz, J.; Cañedo, L. M.; Fernández Puentes, J. L. *J. Antibot.* **1997**, *50*, 738−741.
(38) Traber, R.; Kuhn, M.; Rüegger, A.; Lichti, H.; Loosli, H.-R.; von Wartburg, A. *Helv. Chim. Acta* **1977**, *60*, 1247−1255.
(39) Chatterjee, J.; Gilon, C.; Hoffman, A.; Kessler, H. *Acc. Chem. Res.* **2008**, *41*, 1331−1342.
(40) Doedens, L.; Opperer, F.; Cai, M.; Beck, J. G.; Dedek, M.; Palmer, E.; Hruby, V. J.; Kessler, H. *J. Am. Chem. Soc.* **2010**, *132*, 8115−8128.
(41) Gordon, D. J.; Sciarretta, K. L.; Meredith, S. C. *Biochemistry* **2001**, *40*, 8237−8245.
(42) Lanning, J. D.; Hawk, A. J.; Derryberry, J.; Meredith, S. C. *Biochemistry* **2010**, *49*, 7108−7118.
(43) Malakoutikhah, M.; Prades, R.; Teixidó, M.; Giralt, E. *J. Med. Chem.* **2010**, *53*, 2354−2363.
(44) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243−2266.
(45) Although DTT is the ideal reducing agent regarding its demonstrated biocompatibility, we have also tried to alternatively use TCEP (tris(2-carboxyethyl)phosphine). However, large amounts of desulfurized starting materials were detected by UPLC-MS (see Supporting Information Figure S4). Alternatively to DTT, we have shown that 1,4-butanedithiol can be used with similar half-time of reaction compared to DTT (see Supporting Information Figure S13); this last result shows that if both esters and thioesters can be formed

from DTT during the course of the reaction, only the thioesters are mandatory to promote the exchange reaction.
(46) Lehn, J.-M. *Angew. Chem., Int. Ed.* **2013**, *52*, 2−17.
(47) Moulin, E.; Giuseppone, N. Reactions in Dynamic Self-Assemblies. In *Supramolecular Chemistry: From Molecules to Nanomaterials*; Gale, P., Steed, J., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, 2012; Vol. *4*, pp 1543−1574.
(48) Busseron, E.; Ruff, Y.; Moulin, E.; Giuseppone, N. *Nanoscale* **2013**, *5*, 7098−7140.