Peptide Synthesis Hot Paper

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9-Silafluorenyl Dichlorides as Chemically Ligating Coupling Agents and Their Application in Peptide Synthesis

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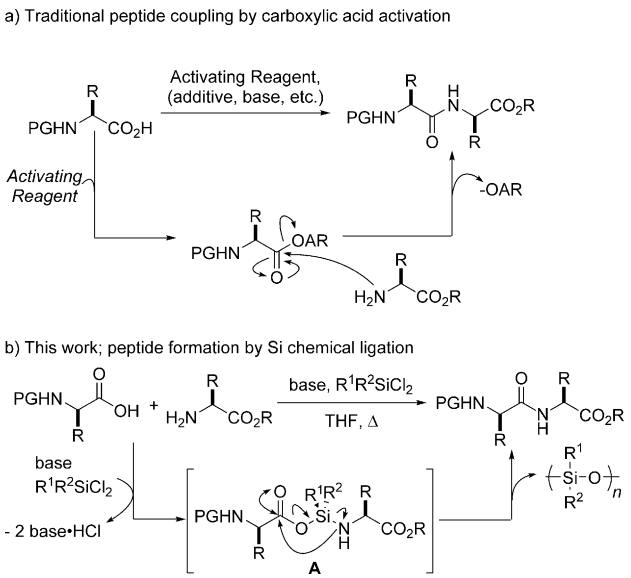
Abstract: *A fundamentally simple, mild, and practical procedure for peptide bond formation is reported that employs a stoichiometric amount of easy-to-access 9-silafluorenyl dichlorides as the coupling agent. Without initial preactivation or elaboration of the carboxylic acid or amine termini of the amino acids, the developed reagent is proposed to act through an unprecedented chemical ligation mechanism, bringing the two coupling partners together before being subsequently eliminated. The desired amides or peptide bonds are thus furnished in good yields and with low to no epimerization.*

The boundless abundance of the amide and peptide bond in pharmaceuticals, biologically active compounds, fine chemicals, and polymers clearly exemplifies their formation as one of the most important reactions in organic chemistry.[1] However, current methods, although general and high-yielding, have inherent limits, including—but not limited to—concerns about waste, expense, and the epimerization of stereocenters. Indeed, to date, most amide and peptide coupling reactions are still carried out using a stoichiometric amount of expensive coupling reagents that can also be potentially hazardous.[2] These methods generally lead to the formation of a large quantity of byproducts, thus imparting inverse environmental effects, and these byproducts need to be separated, sometimes painstakingly, from the final product.[3] In light of this, amide bond formation was recently recognized as a key transformation in which the development of more efficient processes is required.[4]

In recent years, a number of pioneering new strategies have been reported for the synthesis of peptide bonds, such as the catalytic generation of activated carboxylate surrogates from aldehydes,[5] alcohols,[6] and alkynes,[7] the oxidative coupling of α-bromo nitroalkanes with amines,[8] the use of reactive amine surrogates, such as the coupling of isonitriles with carboxylic acids or thioacids,[9] the coupling of thioacids with azides,[10] the coupling of isocyanates with carboxylic acids,[11] and, very recently, the coupling of CDI activated amino esters with carboxylic acids.[12] However, although promising and generally efficient, all of these methods still suffer from the same major limitation: the requirement for prior elaboration of one of the coupling partners to generate a reactive enough species for the formation of the amide bond. One further and particularly promising method that avoids the use of superstoichiometric activating agents is the use of electron-deficient boronic or borinic acids as catalysts to generate an activated carboxylate. First reported in 1996,[13] and with many recent improvements,[14] these methods still generally require the use of a highly functionalized organoboron derivative, forcing conditions, and a dilute reaction medium to achieve good yields.

Herein, we describe an alternative and unprecedented approach to peptide synthesis,[15] involving the synthesis and use of a range of previously unreported 9-silafluorenyl dichlorides as highly effective new coupling reagents. Unlike with traditional methods, no preactivation of either amino acid unit is required (Scheme 1 a). Instead, we propose to chemically ligate[16] both amino acid subunits through a temporary silicon tether **A**, which should rearrange upon heating to produce the desired amide bond by extrusion of an inert siloxane. The driving force for this transformation should be the strength of the newly formed Si–O and amide bonds (Scheme 1 b).



Scheme 1. Traditional and silicon-mediated chemical ligation approaches. AR = activating reagent, PG = protecting group.

We began our work by examining a simple amidation reaction between glycine methyl ester hydrochloride salt and phenylacetic acid to yield amide **1**, employing commercially available dichlorosilanes as coupling agents. To our delight,

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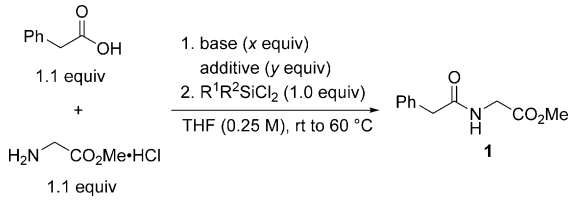
Table 1: Initial optimization of the reaction conditions.[a]

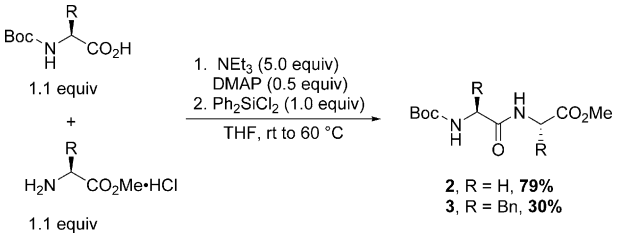


Entry	Dichlorosilane	NEt_3 [equiv]	DMAP [equiv]	Yield[a] [%]
1	Me_2SiCl_2	5.0	–	trace
2	$MePhSiCl_2$	5.0	–	26
3	Ph_2SiCl_2	5.0	–	62
4	Ph_2SiCl_2	4.0	–	46
5	Ph_2SiCl_2	6.0	–	54
6	Ph_2SiCl_2	5.0	0.2	59
7	**Ph_2SiCl_2**	**5.0**	**0.5**	**75**
8	Ph_2SiCl_2	5.0	1.0	56

[a] Determined by ^1H NMR spectroscopy using triphenylmethane as an internal standard.

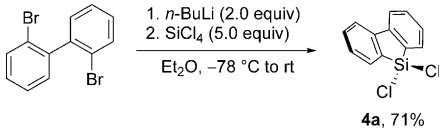
after a short optimization (Table 1), we found that the desired product was formed in up to 75% yield when employing diphenyldichlorosilane as the limiting reagent, an important point to note when addressing the problem of waste in peptide synthesis, along with 5 equivalents of triethylamine and a catalytic amount of DMAP.[17]

With our fundamentally simple proof of concept in hand, we attempted to apply our method directly to the formation of dipeptides. We first decided to couple Boc-Gly-OH and H_2N-Gly-OMe·HCl and were pleased to find that this reaction occurred to yield dipeptide **2** in a gratifying 79% yield. However, when the more sterically challenging coupling of Boc-L-Phe-OH and H_2N-L-Phe-OMe·HCl was attempted, the reaction yield dropped significantly, and **3** was obtained in a moderate 30% yield, although, satisfyingly, no evidence of epimerization was observed by ^1H NMR spectroscopy (Scheme 2).



Scheme 2. Initial peptide coupling reactions employing Ph_2SiCl_2 as the coupling agent. Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine.

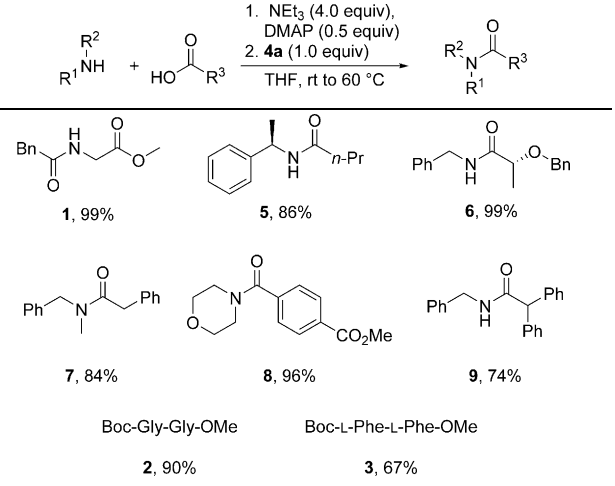
We hypothesized that steric interactions between the bulky amino acid residues and the dichlorosilane were clearly hindering the approach of both coupling partners around the silicon center. We thus looked into modification of the silane coupling agent, and proposed that the use of conformationally flat and rigid 9-silafluorenyl dichloride, compound **4a**, may provide a reagent of similar electronic properties and allow for more facile approach of both amino acid fragments, giving rise to higher yields for bulkier coupling partners. Although not commercially available, this dichlorosilane could be easily synthesized in one step from 2,2′-dibromobiphenyl and $SiCl_4$ in good yield and purity (Scheme 3).[18]



Scheme 3. Synthesis of 9-silafluorenyl dichloride.

Once we had this reagent in hand, it was employed in the initially optimized amidation reaction between H_2N-Gly-OMe·HCl and phenylacetic acid, giving rise to the desired amide product in quantitative yield. Having thus identified a highly efficient silane dichloride coupling partner for the amidation of simple amines with carboxylic acids, we decided to explore the generality of this reaction by subjecting a small range of primary and secondary amines and carboxylic acids to the developed reaction conditions (Table 2).

Table 2: Selected amidation reactions.[a]



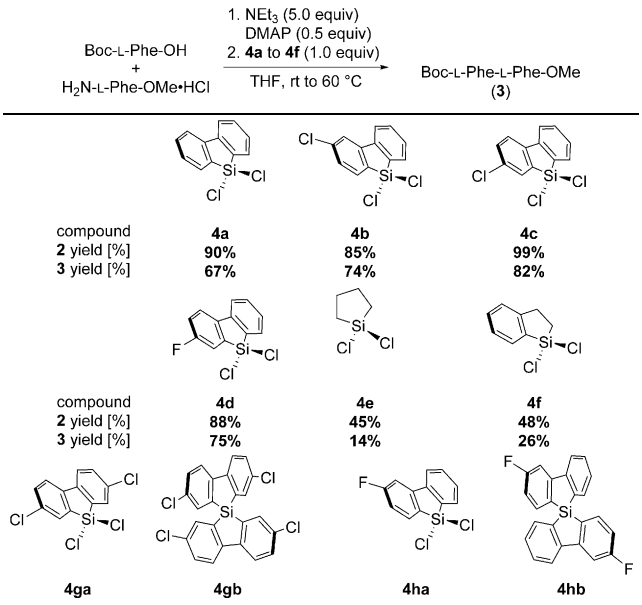
[a] Yields of isolated products are given.

In all cases, the desired secondary (**1**, **5**, **6**, and **9**) and tertiary amide products (**7** and **8**) were all furnished in good to excellent yields. Importantly, supercritical fluid chromatography (SFC) traces showed that no epimerization was observed in the cases where either coupling partner contained a stereocenter (**5** and **6**; see the Supporting Information). Having established that secondary and tertiary amides could be synthesized in good yields, and with no trace of epimerization of the stereocenters, we turned our attention to the more challenging transformation of peptide coupling. Again, the coupling of Boc-Gly-OH with H_2N-Gly-OMe·HCl and that of Boc-L-Phe-OH with H_2N-L-Phe-OMe·HCl were chosen as model reactions. In both cases, a marked increase

in reaction yield was observed, to 90% for the formation of the Boc-Gly-Gly-OMe dipeptide (**2**) and to 67% for Boc-L-Phe-L-Phe-OMe (**3**; Table 2). This represents a great improvement on our method, although still not providing yields high enough to compete with current methods for the synthesis of these dipeptides.

We reasoned that the reactivity of our 9-silafluorenyl dichloride coupling agent could be further tuned through the addition of electron-withdrawing substituents to the silafluorenyl backbone, thus rendering our silane more electrophilic towards the coupling partners. Silanes **4b** to **4f**, bearing chlorine or fluorine substituents on the backbone of the silanyl dichloride, were thus synthesized by dilithiation of the corresponding 2,2′-dibromobiaryls and quenching with $SiCl_4$, and subsequently tested for their activity as coupling reagents in our model peptide coupling reactions (Table 3). In addition, silanes **4e** and **4f**, lacking either one or both of the aryl groups from the fluorenyl backbone, were also synthesized through quenching of their Grignard adducts with $SiCl_4$ and tested in the peptide coupling. Worth noting is that several attempted syntheses of silafluorenyl dichlorides **4ga** and **4ha** yielded inseparable mixtures of both the desired product and sila-spiro compounds **4gb** and **4hb**, and they were not deemed to be viable coupling reagents owing to their seemingly difficult synthesis.

Table 3: Synthesized dichlorosilanes and their activities as peptide coupling agents.[a]



[a] Yields of isolated products are given.

Screening of these new halo-substituted 9-silafluorenyl dichlorides (**4b**–**4d**) for the formation of the sterically challenging Boc-L-Phe-L-Phe-OMe dipeptide (**3**) revealed a clear enhancement of reaction yield when compared to unsubstituted 9-silafluorenyl dichloride (Table 3). Importantly, silane **4c**, bearing a chlorine atom in the 3-position of the fluorenyl backbone, gave an enhanced yield (82%) compared to silane **4b**, which bears a chlorine in the 2-position (74%). This result suggests that this configuration provides the optimal steric and electronic properties to promote a clean and efficient reaction. Surprisingly, the use of silafluorenyl dichloride **4d**, bearing a more electron-withdrawing fluoride substituent in the 3-position, resulted in a diminished yield (75%) compared to its chloride congener. This could be due to the occurrence of side reactions, giving rise to a mixture of side products, or perhaps due to an increase in Si–Cl bond strength, rendering the silane coupling agent less reactive. Silanes **4e** and **4f**, which lack the rigid fluorenyl backbone, highlight its importance, yielding the desired dipeptide (**3**) in just 14% and 26%, respectively. As silane **4c** was previously unreported, an X-ray crystal structure was sought in order to irrefutably prove its structure (Figure 1; see the Supporting Information for the X-ray crystallographic data).

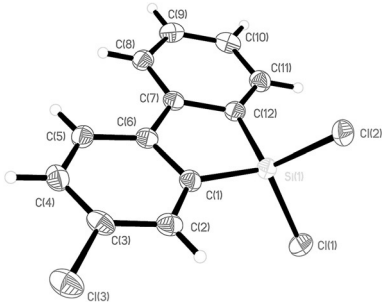
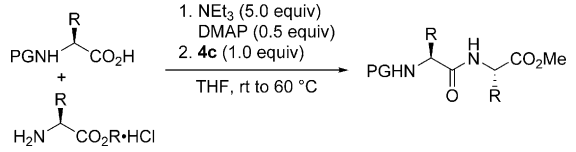


Figure 1. X-ray crystal structure of silane **4c**. Ellipsoids set at 50% probability.[21]

With the optimal 3,5,5-trichlorosilafluorenyl reagent **4c** and reaction conditions in hand, we next submitted a range of monoprotected amino acid coupling partners to our developed method (Table 4). Under the optimized conditions (**4c**, NEt_3, DMAP), all peptides were obtained in good to excellent yields. All reactions proceeded cleanly, with only a filtration and short column chromatography over silica gel being required for purification. The coupling efficiency seemed, however, to be somewhat affected by the amino acid α-side chain. Entries 3 and 4 show a drop in reaction yield from 82% for the coupling of Boc-L-Phe-OH with H_2N-L-Phe-OMe·HCl to 61% for the coupling of bulkier Boc-L-Val-OH with H_2N-L-Phe-OMe·HCl, and entries 3 and 5 show an improvement to 89% yield when smaller Boc-L-Ala-OH was coupled with H_2N-L-Phe-OMe·HCl, as may be expected. Although not conclusive, entries 5 and 6 may give some insight into the order of addition of the coupling partners to the silyl dichloride reagent. Dipeptide **12**, with the bulkier α-side chain on the N-unprotected amino ester, was obtained in higher yield (89%) than dipeptide **13** (81%), where the bulkier α-side chain is present on the free carboxylic acid amino acid residue. This suggests that the first addition to the silyl coupling reagent is that of the free amine residue, and the second, more challenging addition, is that of the free carboxylic acid residue. Importantly, the method was also found to be fully compatible with both Boc and Cbz protecting groups, although with some loss in yield when

Table 4: Scope of the peptide coupling reaction with dichlorosilane **4c**.



Entry	Peptide	Yield[a] [%]
1	Boc-Gly-Gly-OMe (**2**)	99
2	Boc-Gly-L-Phe-OMe (**10**)	91
3	Boc-L-Phe-L-Phe-OMe (**3**)	82
4	Boc-L-Val-L-Phe-OMe (**11**)	61
5	Boc-L-Ala-L-Phe-OMe (**12**)	89
6	Boc-L-Phe-L-Ala-OMe (**13**)	81
7	Boc-L-Met-L-Phe-OMe (**14**)	77
8	Boc-L-Ser(OBn)-L-Ala-OMe (**15**)	82
9	Boc-L-Trp-L-Ala-OMe (**16**)	69
10	Boc-L-Asp(OBn)-Gly-OMe (**17**)	86
11	Boc-L-Pro-Gly-OMe (**18**)	66
12	Boc-L-Pro-L-Ala-OMe (**19**)	65
13	Boc-L-Pro-L-Phe-OMe (**20**)	81
14	Cbz-L-Ala-L-Phe-OMe (**21**)	78
15	Boc-Gly-L-Phe-L-Phe-OMe (**22**)	72
16	Boc-Gly-L-Phe-L-Phe-OMe (**22**)[b]	85
17	Boc-L-Phg-Gly-OMe (**23**)	64

[a] Yields of isolated products are given. [b] Synthesized using Boc-Gly-L-Phe-OH and H_2N-L-Phe-OMe·HCl under the standard conditions. Cbz = benzyloxycarbonyl, PG = protecting group.

switching from *N*-Boc to *N*-Cbz amino acids (entries 5 and 14). More challenging amino acid residues with longer, heteroatom-containing side chains were also found to be well tolerated under our developed conditions, with dipeptides **15** to **17** being furnished in good yields. Finally, we wished to investigate whether our method could be extended to longer-chain peptide residues. As a proof of concept, Boc-Gly-L-Phe-L-Phe-OMe (**22**) was synthesized from H_2N-L-Phe-L-Phe-OMe in 72% yield. The same tripeptide was also synthesized in 85% yield in an N to C pathway using Boc-Gly-L-Phe-OH and H_2N-L-Phe-OMe·HCl (entry 16). In this case, the often problematic diketopiperazine side product was not observed, and the desired tripeptide was delivered in a higher yield. Finally, Boc-L-Phg-OH was used for the synthesis of dipeptide **23** (entry 17) to check for a possible epimerization with such sensitive substrates. Chiral LC analysis gave an enantiospecificity (es) of 86%.[19] Importantly, in all cases, the crude mixtures displayed a remarkable purity according to ^{1}H NMR spectroscopy, the polymeric siloxane byproduct being removed by simple filtration along with the ammonium salts.[20]

A series of in situ monitoring experiments were carried out to identify the putative intermediate **A** (NMR, MS) but we have been unable to unambiguously confirm its formation. However, a series of control experiments support the proposed mechanism (see the Supporting Information for details).[20]

In conclusion, a range of new 9-silafluorenyl dichlorides were synthesized and successfully applied in an unprecedented manner in peptide coupling reactions, with good to excellent yields and limited epimerization on highly sensitive residues only. To the best of our knowledge, this is the first time that such reagents have been applied to these kinds of transformations through a proposed chemical ligation mechanism. The findings of this study provide a strong proof of concept that 9-silafluorenyl dichlorides can be efficiently used in peptide coupling with promising results and minimal waste. Work is currently ongoing in our laboratory towards a related catalytic version of these ligating transformations under smoother conditions.

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[19] LC traces of both the starting material and the final compound **23** can be found in the Supporting Information.

[20] As a representative example, the ^{1}H NMR spectrum of the crude mixture for compound **22** can be found in the Supporting Information.

[21] CCDC 1507219 (**4c**) contains the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.

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