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Stereocontrolled solid-phase synthesis of fluorinated partially-modified retropeptides via tandem aza-Michael/enolate-protonation

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Abstract—*N*-Acylation of Wang resin-bound L-α-amino acids with 2-trifluoromethyl-propenoyl chloride, followed by asymmetric tandem aza-Michael/enolate-protonation by a series of L-α-amino esters and final release from the resin, afforded a representative library of partially-modified retropeptides incorporating a stereodefined trifluoroalanine surrogate. The stereocontrol can be dramatically improved (up to 15:1) by using apolar solvents like carbon tetrachloride and DABCO as base.

Peptide backbone modification is an effective tool for improving bio-stability and selectivity of natural peptides.[1] Partially modified retro peptides (PMR-peptides) represent a particular family of backbone modified peptides (Fig. 1), which is becoming increasingly important in medicinal chemistry, due to the manifold biological activity displayed by a number of them.[2] We have recently described novel peptidomimetic structures incorporating a trifluoromethyl (Tfm) group,[3] within the frame of a research project aimed at developing fluorine-containing protease inhibitors. Recently, we became interested in the development of a solid-phase synthesis of peptide mimics incorporating a stereochemically defined 3,3,3-trifluoroalanine (TF-Ala) unit. However, incorporation of TF-Ala into a peptide sequence **A** (Fig. 1) is extremely difficult,[4] owing to its low chemical and configurational stability at pH>6.[5] Unfortunately, no mimics of TF-Ala-containing peptides have been described until very recently, when we reported a highly stereoselective approach to partially modified retro (PMR) ψ[NHCH$_2$]-peptide mimics **1** (Fig. 1) incorporating a chemically stable and stereodefined [CH$_2$CH(CF$_3$)CO] surrogate of TF-Ala.[6] In order to make available large arrays of PMR-peptides for high throughput biological screening, we decided to study the viability of a *stereocontrolled solid-phase* synthesis of **1**. This goal appeared considerably challenging since the asymmetric synthesis of chiral molecules on solid-phase is still a relatively underdeveloped area.[7] Further challenges were expected owing to the presence of the stereo-electronically demanding Tfm group, and to the strongly acidic nature of the CH(CF$_3$)CO proton, which could lead to epimerization of the stereogenic center under the basic reaction conditions.

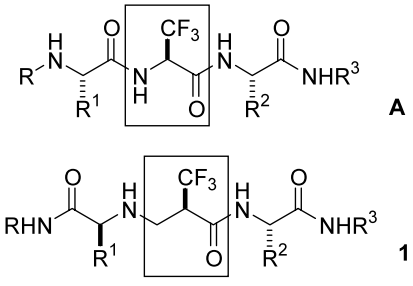

Figure 1. A peptide incorporating L-trifluoroalanine (**A**) and a PMR-ψ[NHCH$_2$]-peptide (**1**) incorporating a stereo-defined trifluoroalanine mimic.

In this communication we describe the efficient stereocontrolled solid-phase synthesis of PMR ψ[NHCH$_2$]-tripeptide mimics, through asymmetric tandem

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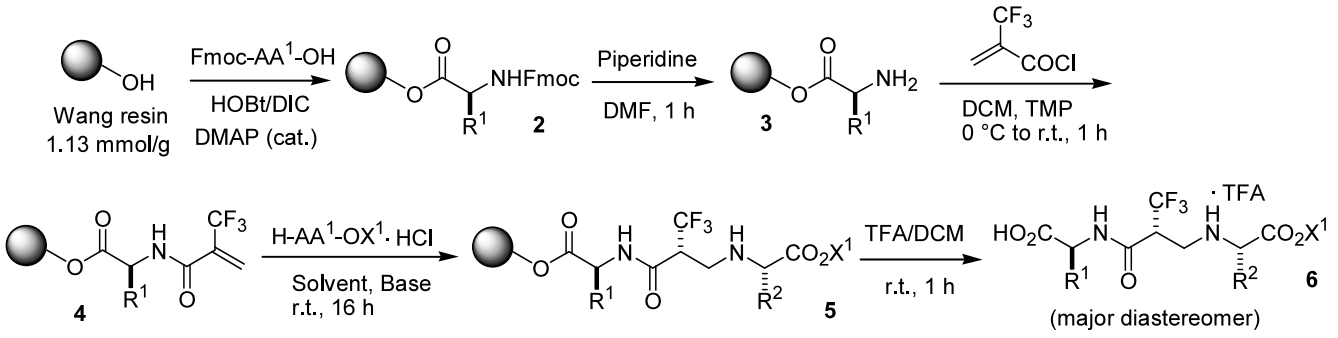
aza-Michael/enolate-protonation of α-substituted α-amino-esters with chiral Wang resin-supported *N*-(2-Tfm)-propenoyl-α-amino ester acceptors.

In the first step (Scheme 1), Wang resin was loaded with *N*-Fmoc α-amino acids to afford the Fmoc-resins **2**, which were *N*-deprotected to **3** upon treatment with piperidine/DMF. Next, the resins **3** were reacted with an excess of 2-trifluoromethyl-propenoyl chloride for 1 hour in dichloromethane (DCM).[8] This process provided the trifluoromethylated resins **4**, functionalized as chiral Michael acceptors.[9] Progress of the coupling could be monitored by FT-IR, which showed a new strong CO band at 1685 cm^{-1}. The crucial aza-Michael reactions were performed by addition of 3 equiv. of the appropriate α-amino ester (see Table 1) to a suspension of resin **4** in the appropriate solvent, in the presence of 6 equiv. of base. The mixtures were shaken at rt for 16 hours, producing the desired resins **5** in a very effective manner.[10] Also in this case the reactions could be monitored by FT-IR of the resin, since appearance of the new CO band considerably broadened the carbonyl region between 1740 and 1685 cm^{-1}. Release of the PMR peptides **6** from the solid support was achieved upon treatment of **5** with TFA in DCM. The target compounds **6** were invariably obtained with good to excellent chemical purity (see Fig. 2). Several highly pure crystalline **6** could be obtained by slow evaporation (24–48 h) of resin-released samples dissolved in methanol.

First of all, we investigated the effect of the amine catalyst on diastereoselectivity conducting model reactions in DCM between polymer supported L-Val acceptor **4a** and L-Val-OBn (Table 1, entries 1–3). The best diastereocontrol was obtained with DABCO (entry 3) which provided **5a** in 6.3:1.0 ratio, as ascertained on the released peptidomimetic **6a** after treatment with TFA/DCM, while lower ratios were obtained with TEA (entry 1) and TMP (entry 2). The effect of solvent was investigated next (entries 3–6), using DABCO as the base of choice. It clearly appears that the solvent is the



Scheme 1. Solid-phase synthesis of PMR $\psi[NHCH_2]$-tripeptide mimics **6** incorporating a trifluoroalanine surrogate.

Table 1. Influence of base and solvent

Entry	Product	Acceptor	R^1	R^2	X^1	Base, solvent	Diast. ratio.	Purity[a]
1	**6a**	**4a**	*i*-Pr	*i*-Pr	Bn	DCM, TEA	4.0:1.0	>95
2	**6a**	**4a**	*i*-Pr	*i*-Pr	Bn	DCM, TMP	4.4:1.0	92
3	**6a**	**4a**	*i*-Pr	*i*-Pr	Bn	DCM, DABCO	6.3:1.0	>95
4	**6a**	**4a**	*i*-Pr	*i*-Pr	Bn	THF, DABCO	4.8:1.0	>95
5	**6a**	**4a**	*i*-Pr	*i*-Pr	Bn	Toluene, DABCO	8.7:1.0	>95
6	**6a**	**4a**	*i*-Pr	*i*-Pr	Bn	CCl_4, DABCO	15.0:1.0	>95
7	**6b**	**4a**	*i*-Pr	*i*-Pr	*t*-Bu[b]	DCM, DABCO	4.8:1.0	90
8	**6c**	**4a**	*i*-Pr	*i*-Bu	Bn	DCM, TMP	5.8:1.0	>95
9	**6d**	**4a**	*i*-Pr	Bn	*t*-Bu[b]	DCM, DABCO	3.3:1.0	89
10	**6e**	**4a**	*i*-Pr	Me	Me	DCM, DABCO	2.1:1.0	93
11	**6f**	**4a**	*i*-Pr	H	Et	DCM, DABCO	1.7:1.0	92
12	**6g**	**4b**	Bn	*i*-Pr	Et	DCM, TMP	3.4:1.0	91
13	**6h**	**4b**	Bn	*i*-Pr	Bn	CCl_4, DABCO	4.7:1.0	77
14	**6i**	**4b**	Bn	Me	Me	DCM, TMP	1.5:1.0	85
15	**6i**	**4b**	Bn	Me	Me	CCl_4, DABCO	3.3:1.0	70
16	**6j**	**4b**	Bn	*i*-Bu	Bn	CCl_4, DABCO	4.5:1.0	73
17	**6k**	**4c**	Me	Ph	Me	DCM, TMP	3.0:1.0	90
18	**6l**	**4c**	Me	*i*-Pr	Bn	CCl_4, DABCO	1.9:1.0	75
19	**6m**	**4c**	Me	*i*-Bu	Bn	CCl_4, DABCO	2.6:1.0	75
20	**6n**	**4c**	Me	Me	Me	CCl_4, DABCO	3.4:1.0	74

[a] Determined by ^1H and ^{19}F NMR of crude compounds **6**, after release from the resin. As a general trend, the ^{19}F NMR signals of the major diastereomers **6** resonate at higher fields than those of the corresponding minor diastereomers.

[b] $X^1 = H$ in **6b**,**d**.

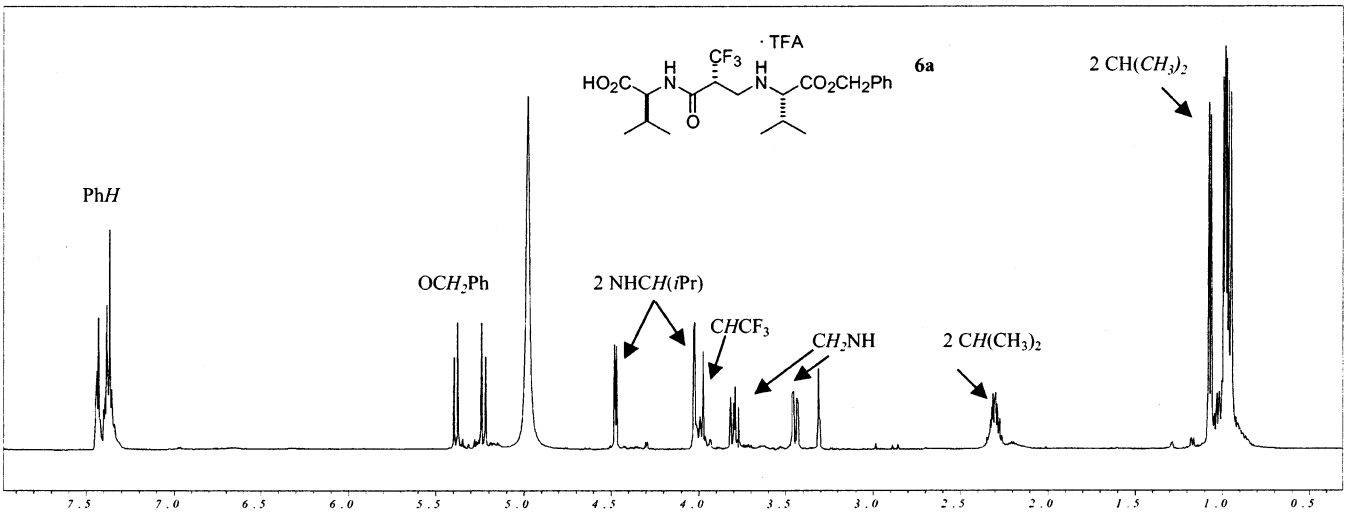


Figure 2. ^{1}H NMR (CD_3OD, 500 MHz) of **6a** obtained under highly stereoselective conditions of entry 6 (Table 1) after release from the resin.

other main factor in this reaction, with low-polarity or apolar solvents providing much higher diastereocontrol. In fact, THF (entry 4) provided lower de than DCM, while better results were achieved with toluene (entry 5). The best diastereoselectivity was finally achieved using apolar CCl_4 (entry 6), which provided **6a** in an excellent 15:1 ratio. Ancillary control experiments also demonstrated that, as expected, the reaction is kinetically controlled, and that, under the optimized conditions (DABCO, CCl_4, rt), the CF_3-substituted stereogenic center of the products **6** is configurationally stable.

Full scope of the methodology in the solid-phase synthesis of PMR-peptides **6**, and effect of the structure of the reactants, namely the polymer-bound acceptors **4** and the α-amino-ester nucleophiles on diastereoselectivity, were investigated next. To this end, a variety of α-amino ester nucleophiles were reacted with the acceptors **4a**–**c**, providing a library of molecules **6b**–**n**, generally with good to excellent yields and purity (entries 7–20, Table 1). Several combinations of solvents and bases were used in these experiments too. The superior performance of CCl_4/DABCO in terms of diastereocontrol was confirmed although better purities were achieved with DCM/TMP (see for example entries 13 versus 12, and 15 versus 14).[11] Both the R^1 side-chain of the polymer supported acceptors **4** and the R^2 side-chain of the α-amino ester nucleophiles have a strong influence on the diastereocontrol. As a rough trend, the de of the products **6** increased with increasing the bulk of the R groups.

The configuration of some PMR-peptides **6** was unambiguously determined by chemical correlation with the analogous compounds synthesized in solution. Alternatively, the configuration was confidentially assigned by assuming that both the solid- and the solution-phase reactions feature the same stereochemical outcome.[6]

In general, one can say that the main features of the solution-phase process[6] were retained in the solid-phase version. The main difference is that the solid-phase reactions in Table 1 were less diastereoselective than those in solution. This could be due to the fact that the polymeric support biases the reaction partners in a different transition state with respect to that assumed in the highly stereoselective solution-phase process. However, this drawback is counterbalanced by the much greater potential of this solid-phase version for a fast, automated generation of large arrays of PMR-ψ[$NHCH_2$]-peptides **1** for high throughput assays, and for the synthesis of polypeptide mimetics as well.

In conclusion, we have described the stereocontrolled solid-phase synthesis of a library of PMR- ψ[$NHCH_2$]-peptides **1** incorporating a stereochemically defined and stable TF-Ala mimic by means of a tandem asymmetric aza-Michael/enolate-protonation. This reaction shows remarkable stereodirecting solvent- and base-effects, which deserve further in depth mechanistic investigations.

Acknowledgements

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9. General experimental procedure for the preparation of resins **4**. To a suspension of resin **3** in dry DCM neat *sym*-collidine (1 equiv. based on the theoretical loading of **3**) followed by a solution of 2-trifluoromethyl propenoyl chloride (3 equiv.) in dry DCM were added under nitrogen atmosphere at 0°C. The suspension was stirred for 1 hour at rt and then filtered. The resin **4** was washed with DMF (×3) and DCM (×5), then the residual solvent was removed at reduced pressure.
10. General experimental procedure for the preparation of resins **5**. To a suspension of resin **4** in dry CH_2Cl_2 solid α-aminoester (3 equiv. based on the theoretical loading of **4**) followed by *sym*-collidine (6 equiv.) were added at rt. The mixture was kept under stirring during 16 hours, then filtered and the resin **5** washed with DMF (×3), CH_2Cl_2 (×3), and MeOH (×3), then the residual solvent was removed at reduced pressure.
11. The reactions performed with the DABCO/CCl_4 system afforded products **6b–n** with slightly lower purities than those featured by the TMP/DCM system.