Synthesis and evaluation of 2-(2-fluoro-4-hydroxymethyl-5-methoxy-phenoxy)acetic acid as a linker in solid-phase synthesis monitored by gel-phase ^{19}F NMR spectroscopy†

Fredrik K. Wallner,‡[a] Sara Spjut,‡[a] Dan Boström[b] and Mikael Elofsson*[a]

Received 23rd March 2007, Accepted 12th June 2007
First published as an Advance Article on the web 28th June 2007
DOI: 10.1039/b704472k

Gel-phase ^{19}F NMR spectroscopy is a useful monitoring technique for solid-phase organic chemistry due to the high information content it delivers and swift acquisition times, using standard NMR spectrometers. This paper describes the synthesis of the novel linker 2-(2-fluoro-4-hydroxymethyl-5-methoxy-phenoxy)acetic acid in 29% yield over seven steps, using nucleophilic aromatic substitutions on 2,4,5-trifluorobenzonitrile as key steps. Following standard solid-phase synthesis a peptide could be cleaved from the linker using 20% TFA in CH_2Cl_2 in 30 minutes, in contrast to a previously described monoalkoxy linker that requires 90% TFA in water at elevated temperature. A resin-bound peptide could be successfully glycosylated using only two equivalents of a thioglycoside donor, activated with *N*-iodosuccinimide and trifluoromethanesulfonic acid, and subsequent cleavage and deprotection gave the target glycopeptide. Direct glycosylation of the linker itself followed by mild acidic cleavage gave a fully protected hemiacetal for further chemical manipulation.

Introduction

Solid-phase syntheses of peptides[1] and oligonucleotides[2] have become standard procedures, with automated methods and synthesizers. Non-peptidic solid-phase organic synthesis is also developing strongly, but mostly for rather simple chemistry with few steps and good regio- and diastereoselectivity. For more complex targets, *e.g.* oligosaccharides, the lack of methods for reaction monitoring still hampers progress.[3] One method that overcomes this problem is gel-phase ^{19}F NMR spectroscopy using fluorine containing linkers, reactants and/or protecting groups. Spectra showing the yield, and in many cases also the diastereomeric ratio, can be recorded in a few minutes using standard NMR spectroscopy equipment. This method has been successfully used in both oligosaccharide and small molecule solid phase organic synthesis.[4–18] In order to accomplish this, several linkers *e.g.* **1**,[17] **2**,[6,15] and **3**[14] (Fig. 1) have been developed that can be cleaved under acidic, basic or oxidative conditions respectively. However, linkers **1** and **2** demand rather harsh conditions for cleavage, such as 90% trifluoroacetic acid (TFA) in water at 60 °C for six hours for **1**.[6,17] Even though more advanced automated or semi-automated synthesizers have the ability to warm and cool the reaction, solid-phase synthesis is greatly simplified if the reactions can be performed at room temperature. To this end, and to further extend the range of linkers and enable more gentle cleavage methods, more acid labile linkers are needed. In non-fluorinated

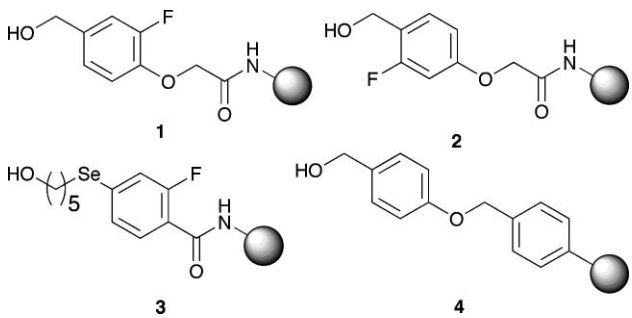


Fig. 1 Examples of fluorinated linkers and the Wang resin **4**. Carboxylic acids attached to **1**, **2**, and **4** are cleavable under acidic or basic conditions, those attached to **3** are cleavable under oxidative conditions.

versions, alkoxy substituted benzyl alcohols have been widely used as linkers, with the classical Wang-linker[19] **4** as the most common one.[20] The Wang-linker is normally cleaved with TFA at room temperature, but the introduction of an electron withdrawing fluorine atom (*cf.* linker **1**) decreases the acid lability of the linker.[6] Increasing the number of electron donating alkoxy groups on the linker conversely increases the acid lability,[20] indicating that the introduction of a methoxy group into the fluorinated linker **1** could give the desired properties. In this study we describe the synthesis and application of the fluorinated dialkoxy linker 2-(2-fluoro-4-hydroxymethyl-5-methoxy-phenoxy)acetic acid.

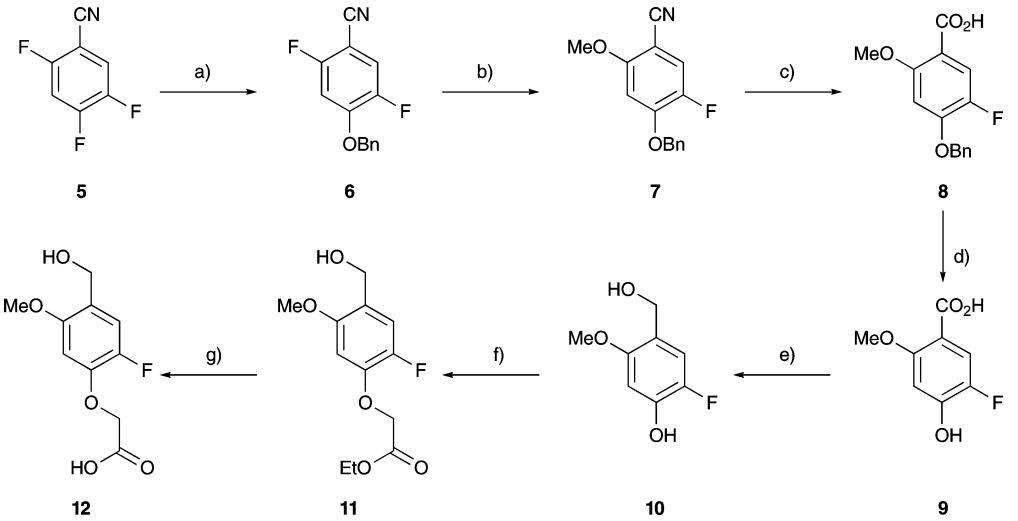
Results and discussion

To synthesize the new linker 2-(2-fluoro-4-hydroxymethyl-5-methoxy-phenoxy)acetic acid **12**, a method based on nucleophilic aromatic substitutions was envisioned. Wells *et al.* demonstrated this method with both *tert*-butyl trifluorobenzoate and trifluorobenzonitrile **5**.[21] Our first strategy was based on the *tert*-butyl trifluorobenzoate pathway with two nucleophilic aromatic

[a]*Department of Chemistry, Umeå University, SE-90187 Umeå, Sweden. E-mail: mikael.elofsson@chem.umu.se; Fax: +46 90 13 88 85; Tel: +46 90 786 93 28*
[b]*Energy Technology and Thermal Process Chemistry, Department of Applied Physics and Electronics, Umeå University, Umeå, Sweden*

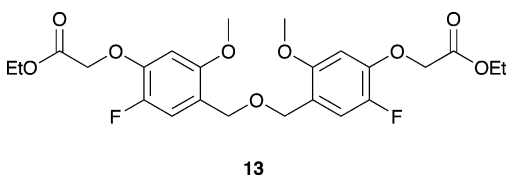
† Electronic supplementary information (ESI) available: Crystal structures and data for **11** and **13** and gel-phase ^{19}F NMR spectra for **18**, **21**, **23**, **26** and **27**. See DOI: 10.1039/b704472k

‡ These two authors contributed equally to this work.



Scheme 1 Synthesis of linker **12**. *Reagents and conditions:* a) t-BuOK, BnOH, THF, −78 °C–room temperature. b) t-BuOK, MeOH, THF, −50 °C–0 °C. c) NaOH, EtOH, reflux. d) H_2 (g), Pd/C, AcOH. e) BH_3·DMS, $B(OMe)_3$, THF. f) Ethyl bromoacetate, DBU, MeCN, reflux. g) LiOH, THF–MeOH–water 3 : 1 : 1.

substitutions as initial steps, first with methyl glycolate in the *para* position and then methoxide in the *ortho* position. The nucleophilic aromatic substitutions, especially the second one, gave low yields due to decomposition of the starting material and transesterification. Instead the linker **12** was prepared from the trifluorobenzonitrile **5** in 29% yield over seven steps (Scheme 1). Nucleophilic aromatic substitutions with potassium benzoxide and methoxide, followed by hydrolysis of the nitrile and hydrogenolysis of the benzyl ether gave the benzoic acid **9** in 53% yield over 4 steps. **9** is an analogue of 3-fluoro-4-hydroxybenzoic acid, the starting material in our previous synthesis of linker **14**.[17] Unfortunately, the increased electron density of **9** disturbed both the reduction to alcohol **10** and the following alkylation. The reduction could however be performed using the same conditions as before in a good yield but the product was very sensitive, most likely due to oxidation, and the product was only briefly characterized before continuation of the synthesis. Alkylation of the phenolic oxygen using ethyl bromoacetate and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) proceeded faster than for the non-alkoxy version. During the reaction the product was surprisingly dimerized on the benzylic position to form **13** (Fig. 2). Since **11** and **13** have very similar spectroscopic properties, and both give the benzylic cation as main ion in LC-MS analysis, X-ray crystallography was used to distinguish them (supporting information§). By reducing the reaction time, the formation of **13** could be suppressed. Finally, basic ester hydrolysis furnished the linker **12** after chromatographic purification. With the linker in hand, studies to confirm its acid lability were performed.



13

Fig. 2 Dimer formed in the alkylation of **10**.

§ CCDC reference numbers 644117 and 644118. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b704472k

Linker **12** and its monoalkoxy analogue **14** were attached to TentaGel HL-NH_2 resin through amide bonds to form linker resins **15** and **1** respectively (Scheme 2). Amides were formed in DMF using *N*,*N*′-diisopropyl-carbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) and monitored with bromophenol blue. Fmoc-glycine was then connected to the benzylic alcohols of **15** and **1** through ester bonds formed with 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) and *N*-methyl-imidazole (MeIm) to give resin **16** and **17**. Continued amide formations with Fmoc-4-fluorophenylalanine and 2,4-difluorobenzoic acid gave the resin-attached peptides **18** and **19**. Carboxylic acids attached to non-fluorinated versions of linker **12** are cleavable with 1% TFA in CH_2Cl_2. Based on this, and the electron withdrawing character of the fluorine atom in **12**, the cleavage study was performed using 5% and 20% TFA in CH_2Cl_2 (Fig. 3).

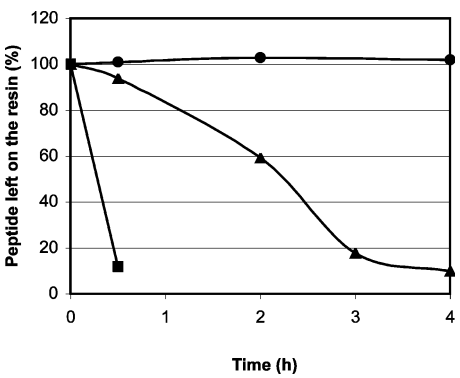
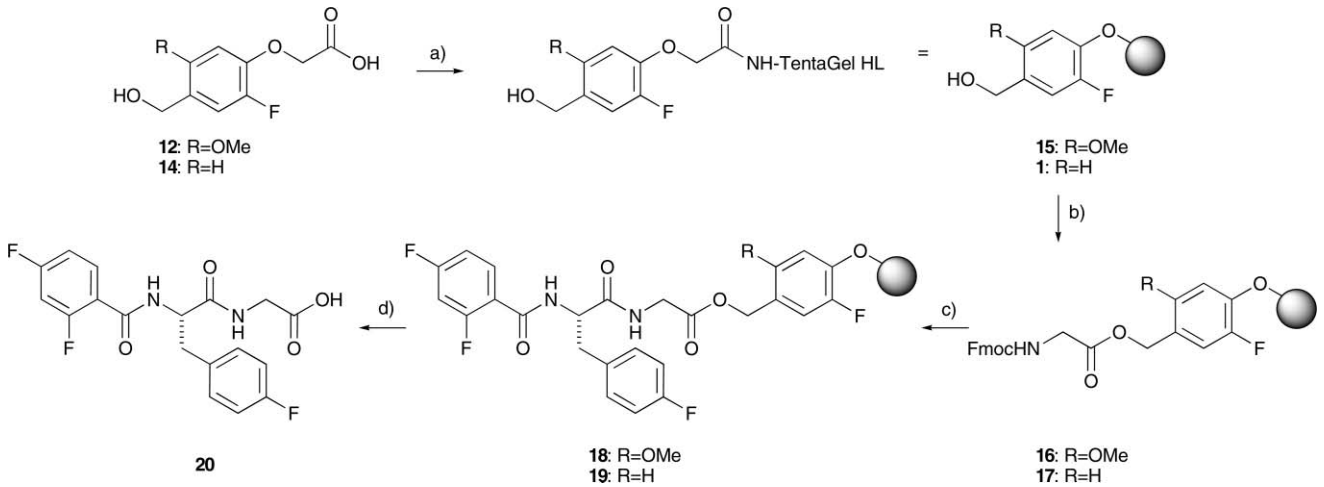


Fig. 3 Cleavage of peptide from resins **19** (black circles) and **18** (black triangles) using 5% TFA in CH_2Cl_2 and **18** (black squares) using 20% TFA in CH_2Cl_2.

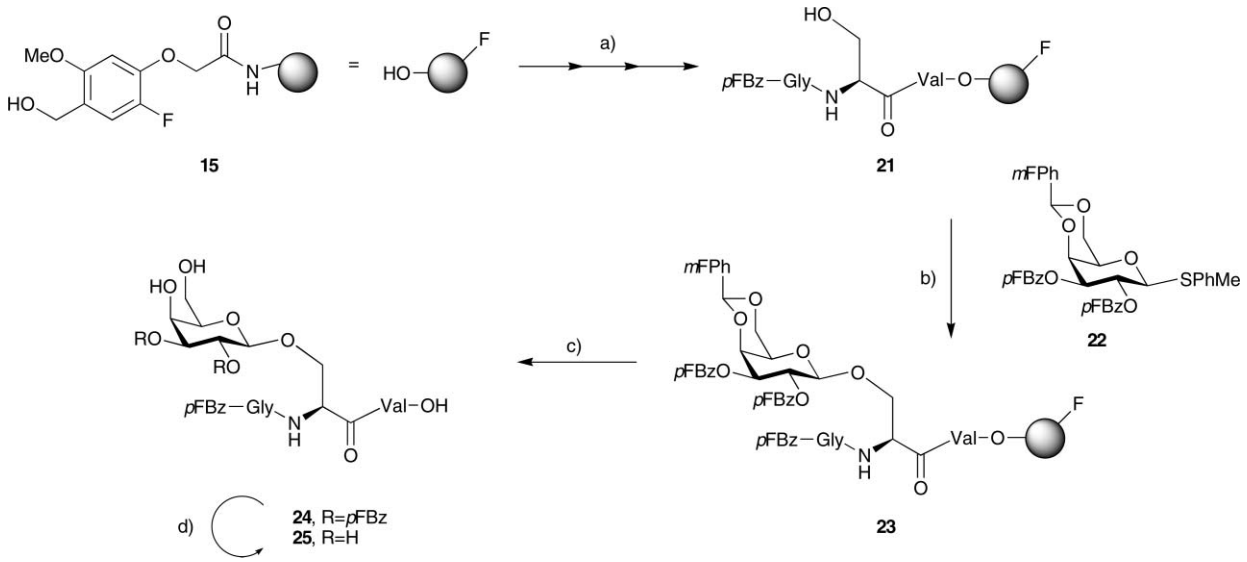
The resins **18** and **19** were treated with the 5% cleavage solution for 30 minutes and gel-phase ^{9}F NMR spectroscopy revealed ~10% cleavage from resin **18** and none from resin **19**. Another



Scheme 2 Synthesis and cleavage of test peptide *Reagents and conditions:* a) TentaGel HL-NH_2, DIC, HOBt, DMF. b) Fmoc-Gly-OH, MSNT, MeIm, CH_2Cl_2. c) i. Piperidine, 20% in DMF. ii. Fmoc-*p*-F-Phe-OH, DIC, HOBt, DMF. iii. Piperidine, 20% in DMF. iv. 2,4-Difluorobenzoic acid, DIC, HOBt, DMF. d) 5% TFA in CH_2Cl_2 (from **18**).

portion of cleavage solution was added and the mixture was shaken for 90 minutes and gel-phase ^{19}F NMR spectroscopy then showed ~40% cleaved from resin **18**. The procedure was repeated and after a total of four hours ~90% was cleaved from resin **18** while resin **19** still was intact. Prolonged treatment for up to seven hours of **19** did not result in any cleavage. When resin **18** was treated with 20% TFA in CH_2Cl_2 for 30 minutes ~90% of the peptide was cleaved. Interestingly, about 5% of the resin bound peptide could not be cleaved with either 5% or 20% TFA despite increased reaction time. This indicates that some of the peptide is bound to the resin matrix in a non-cleavable way. Cleavage of resin **18** with both 5% and 20% TFA in CH_2Cl_2 gave peptide **20** in ~95% yield based on the loading capacity of resin **16**. Resin **19** was then cleaved using the previously described method of TFA–water at 60 °C.[18] Analytical data for **20** was in agreement with those published.[18] In a preliminary study, the Tentagel linker resin **2**, a regioisomer of **14**, seemed to have an acid lability in between **12** and **14** (data not shown).

To test the utility of linker **12** during solid-phase glycosylations, a glycopeptide was assembled. The peptide **21** was prepared as described by Mogemark *et al.*[15] The serine hydroxyl was then glycosylated to give **23** in a remarkable 89% yield using only two equivalents of the galactose donor **22**[15] under promotion by *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) without loss of peptide from the resin (Scheme 3). The yield was determined by comparison of the gel-phase ^{19}F NMR signals from the N-terminal *p*-fluorobenzoyl amide groups with signals from the fluorine containing protecting groups on the glycosyl donor (Fig. 4). From the gel-phase ^{19}F NMR spectra it was concluded that the glycosylation was β-selective since no signals from the



Scheme 3 Synthesis of glycopeptide **25**. *Reagents and conditions:* a) i) Fmoc-Val-OH, MSNT, MeIm, CH_2Cl_2, room temperature, 14 h. ii) Peptide synthesis according to previously reported procedure.[22] b) NIS, TfOH, CH_2Cl_2. c) TFA–water 9 : 1, room temperature, 2 h. d) LiOH (20 mM in MeOH–water 4 : 1).

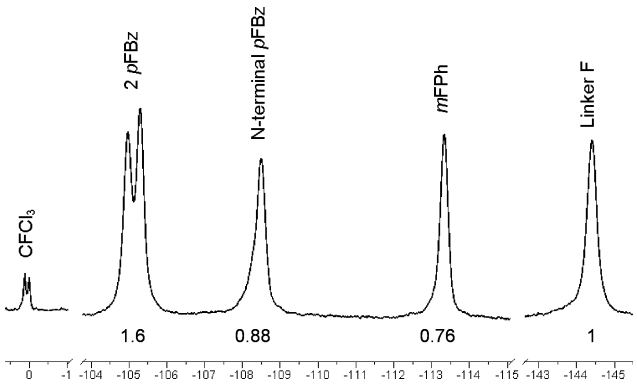
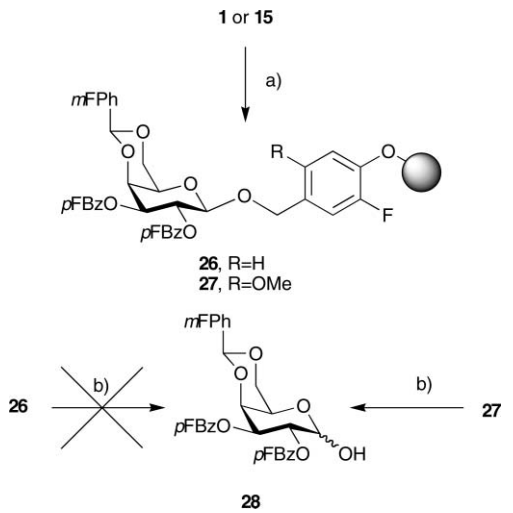


Fig. 4 Gel-phase ^{19}F NMR spectra with integral values for **23**.

α-anomer were seen. Cleavage and simultaneous deprotection of the benzylidene protecting group with TFA–water (9 : 1) at room temperature followed by preparative HPLC gave partially protected glycopeptide **24** in a modest 29% yield based on the resin loading. Debenzoylation with LiOH in MeOH–water (4 : 1) finally yielded glycopeptide **25** in 47% yield.

To test the potential for direct glycosylation the linker resins **15** and **1** were both subjected to four equivalents of galactosyl donor **22**, NIS and a catalytic amount of TfOH (Scheme 4). The donor carries fluorinated versions of common protective groups in carbohydrate chemistry *i.e.* a benzylidene acetal and two benzoates. Examination of the resins with gel-phase ^{19}F NMR spectroscopy revealed that 100% of the more acid stable resin **1** was glycosylated to give **26**, while 40% of the new acid labile linker **15** was glycosylated under the same conditions to give **27**. Resin **27** was obtained in 63% yield after a second glycosylation. The glycosylation gave resin **27** as an anomeric mixture (α : β, 1 : 10) as determined by gel-phase ^{19}F NMR spectroscopy. Obviously resin **15** is more difficult to glycosylate than **1** using our standard protocol. When the glycosylated resins **26** and **27** were treated with the mild cleavage solution 5% TFA in CH_2Cl_2 gel-phase ^{19}F NMR spectroscopy revealed that nothing



Scheme 4 Glycosylation of the linkers **1** and **15**. *Reagents and conditions:* a) 4 or 6 equivalents of **22** and NIS, TfOH (catalytic amount), CH_2Cl_2, room temperature, 4 h or 6 hours. b) 5% TFA in CH_2Cl_2, room temperature, 7 h.

was cleaved from resin **26** while the spectra for resin **27** indicated that approximately 72% was cleaved to give the hemiacetal **28**. Despite another seven hours with the cleavage solution a small amount of the galactose moiety was not cleaved. The isolated total yield of **28** was 58% based on loading capacity of the linker resin **16**. The mild cleavage conditions make it possible to maintain sensitive protecting groups such as benzylidene acetals during the cleavage step from the solid-phase. An attractive strategy would be to assemble oligosaccharides on the linker and the protected hemiacetals obtained after cleavage can be transformed into various donators *e.g.* trichloroacetamides and phosphates for further glycosylations, in a convergent manner. This strategy is currently under investigation in our laboratory.

Conclusions

Gel-phase ^{19}F NMR spectroscopy is a technique well suited for monitoring solid-phase organic synthesis due to its simplicity and sensitivity. Fluorinated linkers with diverse cleavage methods are of great use for this technique. We have prepared a new acid labile fluorinated linker analogue to the commercial 4-hydroxymethyl-phenoxy acetic acid (HMPA) linker. The synthesis was based on nucleophilic aromatic substitutions and the acid lability of the resulting linker was evaluated. It was shown that a test peptide could be cleaved from the resin using 5% TFA in CH_2Cl_2 and after four hours ~90% of the peptide was cleaved. Under the same conditions a previously described linker, an analogue to the Wang-linker, was not affected at all. When the peptide was treated with 20% TFA in CH_2Cl_2 ~90% was cleaved from the new acid-labile linker in only 30 minutes. In addition, the new linker is stable during glycosylation with thioglycosides with NIS–TfOH, as demonstrated by the synthesis of a glycopeptide. It was also shown that the new linker could be directly glycosylated with a thioglycoside using NIS and TfOH as promoters. This implies that the linker can be used for the synthesis of oligosaccharides. The process for glycosylation of the linker however needs to be optimized since six equivalents of glycosyl donor were required. The glycosylation protocol is currently being optimized by our group. The protected hemiacetal was cleaved from the new acid labile resin under mild conditions using 5% TFA in CH_2Cl_2 for a total of 14 hours leaving the benzylidene protective groups intact. The resulting hemiacetal can be transformed into a new glycosyl donor allowing this approach to be employed in a convergent manner. The yield and diastereomeric ratio could easily be determined from the gel-phase ^{19}F NMR spectrum pointing out the value and applicability of this linker for solid-phase glycoconjugate synthesis.

Experimental

Solid-phase synthesis was performed on TentaGel HL-NH_2 resin (0.42 mmol g^{-1}) from Rapp Polymere. CH_2Cl_2 was distilled from calcium hydride, THF from potassium and DMF was distilled under vacuum. Before concentration, all organic solvents were dried over Na_2SO_4 and filtered. Solvent mixtures are reported as v/v ratios. TLC was run on Silica Gel 60 F_{254} (Merck) and the spots were detected in UV-light and stained with phosphomolybdic acid (12 g in 250 ml ethanol) and heat. Silica gel (Matrex, 60 Å, 35–70 μm, Grace Amicon) and solvents of analytical grade were

used for flash column chromatography. The NMR-spectra were recorded on a Bruker DRX-400 or ARX-500 spectrometer with $CDCl_3$, MeOH-d_4 or DMSO-d_6 as solvents and residual $CHCl_3$ (δ_H 7.27 ppm), MeOH-d_3 (δ_H 3.30 ppm) or DMSO-d_5 (δ_H 2.50 ppm) as internal standard for ^1H and $CDCl_3$ (δ_C 77.23 ppm) or MeOH-d_4 (δ_C 49.15 ppm) as internal standard for ^{13}C. $CFCl_3$ (δ_F 0.00 ppm) was added to the solvents as internal standard for ^{19}F. Peaks that could not be assigned are not reported. *J* values are given in Hz. *J* values for the glycopeptide **25** were determined from DQF-COSY. ^{13}C-NMR resonances from the fluorine labeled protective groups are split by J_{C-F} couplings, leading to complex spectra. Since the coupling patterns and hence the actual shift, could not be assigned unambiguously, the peaks are not reported. Gel-phase proton decoupled ^{19}F NMR spectra were recorded on resin suspensions in $CDCl_3$ or MeOH-d_4 with $CFCl_3$ (δ_F 0.00 ppm) as internal standard. Two peaks appear in the spectra around 0 ppm. One originates from $CFCl_3$ inside the polymer and one from $CFCl_3$ outside the polymer. The peak with higher shift was used as internal standard. Preparative reversed phase LC-MS was performed on an XTerra C-18 column (50 × 19 mm, 5 μm, 125 Å), eluted with a linear gradient of MeCN in water, both of which contained formic acid (0.2%). A flow rate of 25 ml min^{-1} was used and detection was at 214 and 254 nm and with positive and negative electrospray mass analysis. Preparative HPLC separations were performed on a Beckman System Gold HPLC, using a Supelco Discovery Biowide Pore C18 column (250 × 212 mm, 5 μm) with a flow rate of 11 ml min^{-1} and detection at 214 nm. Analytical HPLC were performed on a Beckman System Gold HPLC, using a Supelco Discovery Biowide Pore C18 column (250 × 46 mm, 5 μm) with a flow rate of 1.5 ml min^{-1} and detection at 214 nm. Mass spectra were performed on a Waters Micromass ZG 2000 with positive electrospray. Positive fast-atom-bombardment mass spectra were recorded on a Jeol SX102 mass spectrometer. Ions were produced by a beam of Xenon atoms (6 keV) from a matrix of 3-nitrobenzyl alcohol. IR spectra were recorded on an ATI Mattson Infinity Series FTIR™ spectrometer.

4-Benzyloxy-2,5-difluorobenzonitrile (6)

Potassium *tert*-butoxide (1.57 g, 12.9 mmol) was suspended in THF (15 ml) and the mixture was cooled to 0 °C. Benzyl alcohol (2.64 ml, 25.5 mmol) was added and the solution was stirred at 0 °C for 30 min followed by dropwise addition to 2,4,5-trifluorobenzonitrile (2.00 g, 12.7 mmol) in THF (15 ml) at −78 °C. The solution was stirred at −78 °C for 3 h, warmed to room temperature over 1.5 h and stirred at room temperature for 20 h 15 min. The solution was diluted with EtOAc (100 ml) and washed with water (2 × 70 ml). The water phases were reextracted with CH_2Cl_2 (100 ml) and the combined organic phases were concentrated yielding 4.4 g crude product. Recrystallization from EtOH resulted in **6** (2.63 g, 84%). Mp 118–119 °C; ν_{max}/cm^{-1} (solid) 2240 (C≡N); ^1H NMR ($CDCl_3$) δ 7.48–7.36 (m, 5H, C_6H_5), 7.32 (dd, 1H, J = 9.9, 6.0, H-6), 6.84 (dd, 1H, J = 10.0, 6.7, H-3), 5.19 (s, 2H, $PhCH_2$); ^{19}F NMR ($CDCl_3$) δ −108.5 (d, 1F, J = 14.3, F-2), −136.4 (d, 1F, J = 13.3, F-5); ^{13}C NMR ($CDCl_3$) δ 134.6, 129.1, 129.0, 127.6, 71.8; HRMS (FAB) calcd for $C_{14}H_{10}F_2NO$ 2460730 $(M + H)^+$, found 246.0724.

4-Benzyloxy-5-fluoro-2-methoxybenzonitrile (7)

Potassium *tert*-butoxide (1.65 g, 13.5 mmol) was suspended in THF (15 ml), cooled to 0 °C, and methanol (545 μl, 13.5 mmol) was added. The slurry was stirred at 0 °C for 30 min and added to a solution of difluorobenzonitrile **6** (2.36 g, 9.62 mmol) in THF (30 ml) at −50 °C. The mixture was stirred at −50 °C for 1 h, warmed to 0 °C over 30 min, and stirred at 0 °C for 21 h. The solution was diluted with EtOAc (100 ml), washed with water (2 × 100 ml), and the water phases were extracted with CH_2Cl_2 (100 ml). The combined organic phases were concentrated and recrystallization from EtOH gave **7** (1.96 g, 79%). Mp 125 °C; ν_{max}/cm^{-1} (solid) 2213 (C≡N); ^1H NMR ($CDCl_3$) δ 7.49–7.35 (m, 5H, C_6H_5), 7.26 (d, 1H, J = 10.2, H-6), 6.57 (d, 1H, J = 6.7, H-3), 5.22 (s, 2H, $PhCH_2$), 3.85 (s, 3H, CH_3); ^{19}F NMR ($CDCl_3$) δ −142.2; ^{13}C NMR ($CDCl_3$) δ 135.3, 129.0, 128.8, 127.6, 71.7, 56.7; HRMS (FAB) calcd for $C_{15}H_{13}FNO_2$ 258.0930 $(M + H)^+$, found 258.0931.

4-Benzyloxy-5-fluoro-2-methoxybenzoic acid (8)

Benzonitrile **7** (499 mg, 1.94 mmol) was suspended in EtOH (5 ml), NaOH (2 M aq, 3.9 ml, 7.8 mmol) was added and the mixture was refluxed for 39 h and concentrated. The residue was separated between EtOAc and NaOH (1 M aq). The water phase was washed with EtOAc, acidified (pH 1) with HCl (conc. aq), and extracted twice with EtOAc. The combined organic phases from the acidic extraction were concentrated yielding **8** (494 mg, 92%). Mp 126–127 °C; ν_{max}/cm^{-1} (solid) 2942 (OH), 1662 (C=O); ^1H NMR (MeOH-d_4) δ 7.61 (d, 1H, J = 11.8, H-6), 7.50–7.43 (m, 2H, C_6H_5), 7.42–7.30 (m, 3H, C_6H_5), 6.85 (d, 1H, J = 6.8, H-3), 5.26 (s, 2H, $PhCH_2$), 3.87 (s, 3H, CH_3); ^{19}F NMR (MeOH-d_4) δ −144.3; ^{13}C NMR (MeOH-d_4) δ 168.1, 137.7, 129.8, 129.5, 128.9, 72.4, 57.3; HRMS (FAB) calcd for $C_{15}H_{14}FO_4$ 277.0876 $(M + H)^+$, found 277.0870.

5-Fluoro-4-hydroxy-2-methoxybenzoic acid (9)

Benzoic acid **8** (597 mg, 2.16 mmol) and palladium (10% on carbon, 607 mg) were dissolved/suspended in AcOH (300 ml) and stirred under H_2 (g, 1 atm) for 23 h at room temperature. The mixture was filtered through Celite and the solvents were evaporated and coevaporated with MeOH–hexane yielding 0.4 g crude product. The residue was dissolved in EtOAc and washed with water. The water phase was reextracted with EtOAc and the combined organic phases were concentrated yielding **9** (347 mg, 86%). Mp 170 °C; ν_{max}/cm^{-1} (solid) 3220 (OH), 1689 (C=O); ^1H NMR (MeOH-d_4) δ 7.58 (d, 1H, J = 11.7, H-6), 6.62 (d, 1H, J = 7.1, H-3), 3.85 (s, 3H, CH_3O); ^{19}F NMR (MeOH-d_4) δ −147.0; ^{13}C NMR (MeOH-d_4) δ 57.1; HRMS (FAB) calcd for $C_8H_7FO_4$ 187.0407 $(M + H)^+$, found 187.0411.

5-Fluoro-4-hydroxy-2-methoxybenzyl alcohol (10)

Benzoic acid **9** (150 mg, 806 μmol) was dissolved in THF (3 ml) and added slowly to trimethyl borate (0.73 ml, 6.4 mmol) and borane–dimethyl sulfide complex (0.5 ml, 5 mmol) in THF (6 ml). The solution immediately became milky with a white precipitate and was then stirred at room temperature for 24 h. After 4 h the mixture was clear. The reaction was quenched with MeOH (10 ml)

and the solvents were evaporated and the residue was evaporated from MeOH. Flash column chromatography (heptane–EtOAc 2 : 1) yielded **10** (128 mg, 92%) as an oil. ν_{max}/cm $^{-1}$ (oil) 3297 (OH); ^{1}H NMR (MeOH-d_4) δ 7.01 (d, 1H, J = 11.5, H-6), 6.51 (d, 1H, J = 7.3, H-3), 4.49 (s, 2H, $PhCH_2$), 3.75 (s, 3H, CH_3); ^{19}F NMR (MeOH-d_4) δ −148.8; HRMS (FAB) calcd for $C_8H_9FO_3$ 172.1537 $(M)^+$, found 172.0546.

Ethyl (2-fluoro-4-hydroxymethyl-3-methoxyphenoxy)acetate (11)

Phenol **10** (305 mg, 1.77 mmol) and ethyl bromoacetate (395 µl, 3.56 mmol) in MeCN (22 ml) were stirred at room temperature and DBU (400 µl, 2.65 mmol) was added dropwise. The solution was refluxed for 4.5 h. The yellow solution was allowed to reach room temperature, poured into EtOAc (100 ml), and washed with HCl (0.05 M aq, 100 ml) and brine (100 ml). The organic phase was evaporated yielding 0.5 g crude material as a yellow oil. A first chromatography (heptane–EtOAc 3 : 1) gave 0.36 g partly purified material. A second chromatography (CH_2Cl_2–MeOH 100 : 1) gave **11**, sufficiently pure for further use (329 mg, 72%). Mp 47 °C; ν_{max}/cm $^{-1}$ (solid) 3282 (OH), 1766 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.03 (d, 1H, J = 11.6, H-6), 6.54 (d, 1H, J = 6.8, H-3), 4.66 (s, 2H, $C(O)CH_2O$), 4.55 (s, 2H, $PhCH_2$), 4.24 (q, 2H, J = 7.1, CH_3CH_2), 3.76 (s, 3H, CH_3O), 2.57 (br s, 1H, OH), 1.27 (t, 3H, J = 7.1, CH_2CH_3); ^{19}F NMR ($CDCl_3$) δ −143.6; HRMS (FAB) calcd for $C_{12}H_{15}FO_5$ 258.2429 $(M)^+$, found 258.0907.

2-(2-Fluoro-4-hydroxymethyl-5-methoxyphenoxy)acetic acid (12)

Acetate **11** (321 mg, 1.24 mmol) was dissolved in THF–MeOH–water (3 : 1 : 1, 25 ml) and cooled to 0 °C. LiOH (1 M aq, 1.74 ml) was added slowly and the resulting solution was stirred at 0 °C for 30 min. The solution was allowed to reach room temperature and stirred for additional 2 h 15 min. After cooling to 0 °C the solution was acidified with HCl (1 M aq) and extracted with EtOAc (2 × 25 ml). The organic phases were washed with brine (25 ml) and evaporated yielding 0.36 g crude product. Chromatography (heptane–EtOAc 1 : 1 + 2.5% AcOH) gave **12** (233 mg, 81%). Mp 124 °C; ν_{max}/cm $^{-1}$ (solid) 3370 (OH), 1978 (C=O); ^{1}H NMR (MeOH-d_4) δ 7.10 (d, 1H, J = 11.9, H-6), 6.67 (d, 1H, J = 7.0, H-3), 4.72 (s, 2H, $C(O)CH_2O$), 4.52 (s, 2H, $PhCH_2$), 3.79 (s, 3H, CH_3O); ^{19}F NMR (MeOH-d_4) δ −144.5; ^{13}C NMR (MeOH-d_4) δ 172.5, 67.9, 59.6, 56.6; HRMS (FAB) calcd for $C_{10}H_{11}FO_5$ 230.1897 $(M)^+$, found 230.0598.

Resin 1, 17 and 19

Preparation and analytical data in agreement with those previously reported.[18,23]

Resin 15. Tentagel-HL-NH_2 (600 mg, 0.260 mmol) was swelled in distilled DMF (4 ml) and filtered. Linker **12** (88 mg, 0.382 mmol) and HOBt (55 mg, 0.407 mmol) were dissolved in distilled DMF (3 ml) and DIC (61 µl, 0.392 mmol) was added. After stirring at room temperature for 5 min bromophenol blue (2 mM in DMF, 20 µl) was added and the solution was added to the resin. The mixture was mechanically agitated for 15 h (until the mixture became yellow). The resin was washed with DMF and CH_2Cl_2 (5 × 5 ml each). CH_2Cl_2–Ac_2O–pyridine (1 : 1 : 1, 6 ml) was added to the resin and the mixture was agitated mechanically for 5 h. The resin was washed with DMF, CH_2Cl_2, DMF (5 × 5 ml each) and dry MeOH (2 × 5 ml). NaOMe (0.2 M in MeOH, 5 ml, 1.0 mmol) was added to the resin and the mixture was agitated mechanically for 15 h. The resin was washed with DMF (2 × 5 ml), MeOH (5 ml), and CH_2Cl_2 (5 × 5 ml) to give **15**. ^{19}F NMR ($CDCl_3$) δ −144.7.

Resin 16. Fmoc-Gly-OH (232 mg, 0.780 mmol) and MSNT (233 mg, 0.786 mmol) was suspended in dry CH_2Cl_2 (3 ml) and *N*-methylimidazole (MeIm, 60 µl, 0.753 mmol) was added. The mixture was stirred for 5 min until the solution was clear. The solution was added to resin **1** (0.260 mmol), prewashed with dry CH_2Cl_2, and the mixture was agitated mechanically for 22 h. The resin was washed with CH_2Cl_2, DMF (3 × 5 ml each), and CH_2Cl_2 (2 × 5 ml). ^{19}F NMR ($CDCl_3$) δ −144.5. Fmoc determination[24] was performed on **16** in order to determine the loading capacity of the resin bound linker yielding a loading capacity of 0.35 mmol g^{-1} (given as a mean value of three independent measurements) instead of 0.42 mmol g^{-1} given by the supplier.

Resin 18

Resin **16** (211 mmol) was treated with piperidine (20% in DMF, 2 × 7 ml, 5 and 15 min respectively). The resin was washed with DMF (3 × 5 ml) and distilled DMF (3 × 3 ml). Fmoc-*p*F-L-Phe-OH (422 mg, 1.041 mmol) and HOBt (211 mg, 1.562 mmol) were dissolved in distilled DMF (3 ml). DIC (160 µl, 1.027 mmol) was added and the solution was stirred at room temperature for 5 min before bromophenol blue (2 mM in DMF, 20 µl) was added. The solution was added to the resin and the mixture was agitated mechanically for 2 h (until the mixture became yellow). The resin was washed with DMF and CH_2Cl_2 (3 × 5 ml each). ^{19}F NMR ($CDCl_3$) δ −116.4, −144.4. The resin was treated with piperidine (20% in DMF, 2 × 7 ml, 5 and 15 min respectively) and washed with DMF (3 × 5 ml) and distilled DMF (3 × 3 ml). *o*,*p*-Difluorobenzoic acid (165 mg, 1.044 mmol) and HOBt (211 mg, 1.562 mmol) were dissolved in distilled DMF (3 ml). DIC (160 µl, 1.027 mmol) was added and the solution was stirred at room temperature for 5 min before bromophenol blue (2 mM in DMF, 20 µl) was added. The solution was added to the resin and the mixture was agitated mechanically for 1 h 30 min (until the mixture became yellow). The resin was washed with DMF and CH_2Cl_2 (3 × 5 ml each) to give resin **18**. ^{19}F NMR ($CDCl_3$) δ −104.0, −108.8, −116.3, −144.5.

[*N*-(2,4-Difluorobenzoyl)-4-fluorophenylalanyl]glycine (20)

To resin **18** (53 µmol) TFA (5% or 20% in CH_2Cl_2, 2 ml) was added and the mixture was mechanically agitated for 30 min. After extensive washings and ^{19}F NMR spectroscopy the procedure was repeated three times. After a total of 7 h the combined filtrates were concentrated and preparative LC-MS gave **20** (20 mg, 96% based on Fmoc determination[24] of the linker resin **16**). Analytical data in agreement with previously reported.[18]

Resin 21

Preparation and analytical data in agreement with previously reported.[15]

Resin 23

Resin **21** (302 μmol), glycosyl donor **22** (387 mg, 608 μmol) and NIS (140 mg, 622 μmol) were dried under vacuum overnight in the absence of light. Distilled CH_2Cl_2 (4 ml) was added, followed by TfOH (1 μl, 0.01 mmol). The mixture was agitated mechanically for 3.5 h in the absence of light. The resin was washed with CH_2Cl_2, THF, piperidine (20% in DMF), DMF, and CH_2Cl_2 (3 × 15 ml each). Gel-phase ^{19}F NMR spectroscopy indicated that resin **23** was prepared in a yield of 89%. ^{19}F NMR ($CDCl_3$) δ −105.1, −105.4, −108.6, −113.4 and −144.5.

4-Fluorobenzoyl-L-glycyl-*O*-(β-D-galactopyranosyl)-L-seryl-L-valine (25)

A solution of TFA in H_2O (9 : 1, 10 ml) was added to resin **23** (150 μmol). The mixture was agitated mechanically at room temperature for 2 h. The resin was removed by filtration and washed with H_2O, THF and CH_2Cl_2. The filtrate was concentrated. Preparative HPLC yielded the partially deprotected glycopeptide **24** (34 mg, 29%). **24** was dissolved in MeOH (5 ml) and aqueous LiOH (0.81 ml, 0.1 M) was added dropwise. After 1 h the solution was neutralized with AcOH, concentrated, diluted with AcOH (2 ml), and lyophilized. Preparative HPLC yielded **25** (10.8 mg, 47%) with analytical data in agreement with those previously reported[15] except for the data for the galactose hydrogens H-1 to H-4 which instead should be as follows. ^{1}H NMR (DMSO-d_6) Gal: 4.15–4.12 (m, 1H, J = 9.2, H-1), 3.97–3.93 (m, 1H, H-4), 3.64–3.62 (m, 1H, H-3), 3.31–3.28 (m, 1H, H-2) ^{19}F NMR (DMSO-d_6) δ −109.2.

Resin 26

Resin **1** (0.120 mmol) and the glycosyl donor **22** (0.305 g, 0.479 mmol) were put under vacuum for one hour followed by addition of NIS (0.102 g, 0.453 mmol) in the absence of light. Distilled CH_2Cl_2 (4 ml) and a catalytic amount of TfOH were added. The mixture was stirred at room temperature for 3.5 h in the absence of light. The resin was washed with CH_2Cl_2, THF, 20% piperidine in DMF, DMF, and CH_2Cl_2 (3 × 15 ml). According to gel-phase ^{19}F NMR spectroscopy the yield was 100% and only one isomer was formed. ^{19}F NMR ($CDCl_3$) δ −105.0 (s, 1F, 4-*F*PhCO$_2$), −105.4 (s, 1F, 4-*F*PhCO$_2$), −113.5 (s, 1F, 3-*F*PhCHO$_2$), −134.0 (s, 1F, linker *F*).

Resin 27

Resin **15** (0.120 mmol) and the glycosyl donor **22** (0.301 g, 0.473 mmol) were put under vacuum for 1 h followed by addition of NIS (0.108 g, 0.480 mmol) in the absence of light. Distilled CH_2Cl_2 (4 ml) and TfOH (1.0 M in CH_2Cl_2, 0.017 mmol, 17 μmol) were added. The mixture was stirred at room temperature for 4 h in absence of light. The resin was washed with CH_2Cl_2, THF, 20% piperidine in DMF, DMF and CH_2Cl_2 (3 × 15 ml). According to gel-phase ^{19}F NMR spectroscopy the yield was ~40%. When the glycosylation was repeated with glycosyl donor **22** (0.153 g, 0.240 mmol), NIS (0.051 g, 0.227 mmol) and catalytic amount of TfOH (1.0 M in CH_2Cl_2, 0.017 mmol, 17 μmol) for 2 h, the yield was increased to 63%. According to gel-phase ^{19}F NMR spectroscopy two isomers were formed with the ratio 1 : 10. ^{19}F NMR ($CDCl_3$) δ major isomer −105.0 (s, 1F, 4-*F*PhCO$_2$), −104.5 (s, 1F, 4-*F*PhCO$_2$), −113.5 (s, 1F, 3-*F*PhHCO$_2$), −144.6 (s, 1F, linker *F*); minor isomer −105.7 (s, 1F, 4-*F*PhCO$_2$), −106.0 (s, 1F, 4-*F*PhCO$_2$), −113.2 (s, 1F, 3-*F*PhHCO$_2$), −145.0 (s, 1F, linker *F*).

2,3-Di-*O*-*p*-fluorobenzoyl-4,6-*O*-*m*-fluorobenzylidene-D-galactose (28)

The resin **27** (0.060 mmol) was treated with 5% TFA in CH_2Cl_2 for 7 h. The filtrate was washed with CH_2Cl_2 and the procedure was repeated. After extensive washing with CH_2Cl_2 the filtrates were combined and concentrated. Preparative LC-MS gave 15.4 mg **28** (58% total yield based on Fmoc determination of the linker resin **16**). According to NMR spectroscopy two isomers were formed with the ratio 3.8 : 1. ^{1}H NMR ($CDCl_3$) δ 8.11–7.99 (m, 4H, ArH), 7.42–7.24 (m, 3H, ArH), 7.14–7.04 (m, 5H, ArH); major isomer 5.87 (dd, 3.4, 1H, J = 3.4, 10.6, H-3), 5.33–5.75 (m, 2H, H-2, H-1), 5.59 (s, 1H, 3-FPhCHO$_2$), 4.68 (d, 1H, J = 3.3, H-4), 4.36 (dd, 1H, J = 1.2, 12.5, H-6), 4.23–4.15 (m, 2H, H-5, H-6); minor isomer 5.66 (dd, 1H, J = 8.0, 10.3, H-2), 5.60 (s, 1H, 3-FPhCHO$_2$), 5.49 (dd, 1H, J = 3.6, 10.4, H-3), 4.61 (d, 1H, J = 3.4, H-4), 4.48 (dd, 1H, J = 1.4, 12.4, H-6), 3.77 (s-br, 1H, J = 10.2, H-5); ^{19}F NMR ($CDCl_3$) δ major isomer −105.1 (s, 1F, 4-*F*PhCO$_2$), −105.2 (s, 1F, 4-*F*PhCO$_2$), −113.6 (s, 1F, 3-*F*PhHCO$_2$); minor isomer −104.7 (s, 1F, 4-*F*PhCO$_2$), −104.8 (s, 1F, 4-*F*PhCO$_2$), −113.5 (s, 1F, 3-*F*PhCHO$_2$); ^{13}C NMR ($CDCl_3$) δ 99.9, 91.5, 74.4, 69.3, 69.1, 68.9, 62.4; HRMS (FAB) calcd form $C_{27}H_{21}F_3NaO_8$ 553.4358 (M + Na)$^+$, found 553.1089.

Acknowledgements

This work was supported by the Swedish Research Council. We thank Johan Vestergren for practical assistance.

Notes and references

1 G. B. Fields, Z. Tian and G. Barany, in *Synthetic Peptides: A User's Guide*, ed. G. A. Grant, W. H. Freeman and Company, New York, 1992, pp. 77–183.
2 M. H. Caruthers, *Science*, 1985, **230**, 281–285.
3 P. H. Seeberger and W.-C. Haase, *Chem. Rev.*, 2000, **100**, 4349–4393.
4 A. Svensson, K.-E. Bergquist, T. Fex and J. Kihlberg, *Tetrahedron Lett.*, 1998, **39**, 7193–7196.
5 J. M. Salvino, N. V. Kumar, E. Orton, J. Airey, T. Kiesow, K. Crawford, R. Mathew, P. Krolikowski, M. Drew, D. Engers, D. Krolikowski, T. Herpin, M. Gardyan, G. McGeehan and R. Labaudiniere, *J. Comb. Chem.*, 2000, **2**, 691–697.
6 A. Svensson, T. Fex and J. Kihlberg, *J. Comb. Chem.*, 2000, **2**, 736–748.
7 M. Mogemark, M. Elofsson and J. Kihlberg, *Org. Lett.*, 2001, **3**, 1463–1466.
8 Y. Pan and C. P. Holmes, *Org. Lett.*, 2001, **3**, 2769–2771.
9 Y. Pan, B. Ruhland and C. P. Holmes, *Angew. Chem., Int. Ed.*, 2001, **40**, 4488–4491.
10 J. M. Salvino, S. Patel, M. Drew, P. Krowlikowski, E. Orton, N. V. Kumar, T. Caulfield and R. Labaudiniere, *J. Comb. Chem.*, 2001, **3**, 177–180.
11 H. Emtenäs, K. Åhlin, J. S. Pinkner, S. J. Hultgren and F. Almqvist, *J. Comb. Chem.*, 2002, **4**, 630–639.
12 M. Mogemark, M. Elofsson and J. Kihlberg, *ChemBioChem*, 2002, **3**, 1266–1269.
13 M. Mogemark, M. Elofsson and J. Kihlberg, *J. Org. Chem.*, 2003, **68**, 7281–7288.
14 M. Mogemark, L. Gustafsson, C. Bengtsson, M. Elofsson and J. Kihlberg, *Org. Lett.*, 2004, **6**, 4885–4888.
15 M. Mogemark, F. Gårdmo, T. Tengel, J. Kihlberg and M. Elofsson, *Org. Biomol. Chem.*, 2004, **2**, 1770–1776.

16 M. Hourdin, G. Gouhier, A. Gautier, E. Condamine and S. R. Piettre, *J. Comb. Chem.*, 2005, **7**, 285–297.
17 F. K. Wallner, H. A. Norberg, A. I. Johansson, M. Mogemark and M. Elofsson, *Org. Biomol. Chem.*, 2005, **3**, 309–315.
18 F. K. Wallner and M. Elofsson, *J. Comb. Chem.*, 2006, **8**, 150–152.
19 S.-S. Wang, *J. Am. Chem. Soc.*, 1973, **95**, 1328–1333.
20 F. Guillier, D. Orain and M. Bradley, *Chem. Rev.*, 2000, **100**, 2091–2157.
21 K. M. Wells, Y.-J. Shi, J. E. Lynch, G. R. Humphrey, R. P. Volante and P. J. Reider, *Tetrahedron Lett.*, 1996, **37**, 6439–6442.
22 J. Broddefalk, M. Forsgren, I. Sethson and J. Kihlberg, *J. Org. Chem.*, 1999, **64**, 8948–8953.
23 F. K. Wallner and M. Elofsson, *J. Comb. Chem.*, 2006, **8**, 440–440.
24 M. Gude, J. Ryf and P. D. White, *Lett. Pept. Sci.*, 2002, **9**, 203–206.