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Role of the *peri*-effect in synthesis and reactivity of highly substituted naphthaldehydes: a novel backbone amide linker for solid-phase synthesis†

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Handles (linkers) with an aldehyde functionality that permits the anchoring of substrates by reductive amination have, since their first report in the mid-1990s, become widely-used tools in solid-phase synthesis. In the synthesis of peptides, they allow anchoring of the growing peptide chain through a backbone amide, thus giving easy access to *C*-terminal modified or cyclic peptides. Recently, we described two new handles (NAL-1 and NAL-2) with dialkoxynaphthaldehyde core structures. Here, we describe the design, synthesis and properties of a novel trialkoxynaphthalene-based backbone amide linker (NAL-3). The NAL-3 handle is based on a trialkoxynaphthaldehyde (NALdehyde-3) that was synthesized in nine high-yielding steps from 3-methoxyphenylacetic acid in 51% overall yield. The naphthalene ring system was constructed using a regioselective methanesulfonic acid-catalyzed ring-closing reaction. The tetra-substituted naphthalene derivative 1,3,6-trimethoxynaphthalene-2-carbaldehyde (**7**) was selectively demethylated in the 1 position using BBr_3. The selectivity of this reaction is discussed, based on the crystal structures of reactant and product, 1-hydroxy-3,6-dimethoxy-naphthalene-2-carbaldehyde (**8**), and in the context of the *peri*-effect. The new handle was anchored to an aminomethylated poly(styrene) solid support, followed by assembly of a model dipeptide, then a study of the cleavage properties under acidic conditions was carried out. Surprisingly, the trialkoxynaphthaldehyde-based handle proved less acid-labile than the dialkoxynaphthaldehyde handles, and this fact is discussed with respect to handle design.

Introduction

The two defining steps in solid-phase synthesis are the anchoring of the first residue through a handle (linker) to the solid support (resin) and the release of the fully assembled product.[1–3] The handle carries a dual functionality: at one end it allows permanent anchoring to the support, the other end has the functionality of a protecting group, which allows attachment and eventually release of the final product.[4] Acid-labile handles, *i.e.* those able to release the final product with dilute trifluoroacetic acid (TFA) or even milder conditions, remain the most widely used. The acid-lability can to a large extent be correlated with the stability of the carbenium ion, which forms after release of the final product, for leaving groups with comparable electronic properties.‡

Handles with an aldehyde functionality that allows the anchoring of substrates by reductive amination, have, since their first report in the mid-1990s become widely used tools in solid-phase synthesis.[5–7] In the synthesis of peptides, they allow anchoring of the growing peptide chain through a backbone amide, thus giving easy access to *C*-terminal modified or cyclic peptides. This backbone amide linker (BAL) concept was first implemented in a trialkoxybenzyl system, which allowed release of final products by treatment with concentrated TFA.[5] Since then, BAL type handles with monoalkoxy-[8], dialkoxy-[9], and alkoxyhydroxybenzyl,[10] as well as indole[11] and thiophene[12] structures have been reported (Fig. 1).

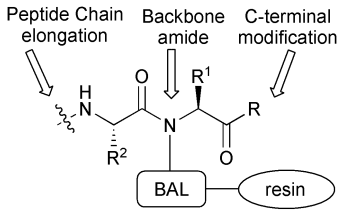

Fig. 1 General backbone amide linkage (BAL) strategy for solid phase synthesis.

The choice of handle for a particular solid-phase synthesis determines the chemistry that can be used for assembly and eventual release of the final product. The development of new handles therefore continues to be important.

Design of naphthalene backbone amide linkers

The goal of our present research is to develop BAL-type linkers with an increased acid-lability. One way of achieving this is to use systems that can further stabilize the carbenium ion formed in the cleavage process. Stabilization of such a carbenium ion can be achieved either by increasing the number of electron donating substituents on the aromatic nucleus,[4] by using electron-rich heteroaromatic compounds[11,12] or by increasing the size of the aromatic system.[13]§ Recently we reported the first two

† Electronic supplementary information (ESI) available: Crystal structures of **7** and **8**, HPLC for Fmoc-Phe-Leu-OH, HMBC spectra of **7**, **8**, **10** and **11**. See http://www.rsc.org/suppdata/ob/b4/b412971g/

‡ The PAL (peptide–amide linker) and BAL (backbone–amide linker) handles generate the same trialkoxybenzyl carbenium ion upon cleavage, however, the PAL handle releases a primary amide, while the corresponding BAL handle releases a secondary amide. Interestingly, the PAL handle requires TFA–H_2O (19 : 1) for release while the BAL handle can release the secondary amide with dilute TFA (as little as 1–2% TFA in CH_2Cl_2). We ascribe this difference to steric factors, possibly steric relief.

§ While trityl-based (*e.g.*, the 2-chlorotrityl chloride) handles are very useful for *C*-terminal anchoring of the growing peptide chain, the steric bulk they impose makes them less suitable for BAL-type anchoring.

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naphthalene based handles, NALdehyde-1 and NALdehyde-2, based on this strategy shown in Fig. 2 (NAL is an acronym for naphthalene backbone amide linker).[14] Here we describe a new trialkoxynaphthaldehyde derivative (NALdehyde-3)¶ and its application in solid-phase synthesis.

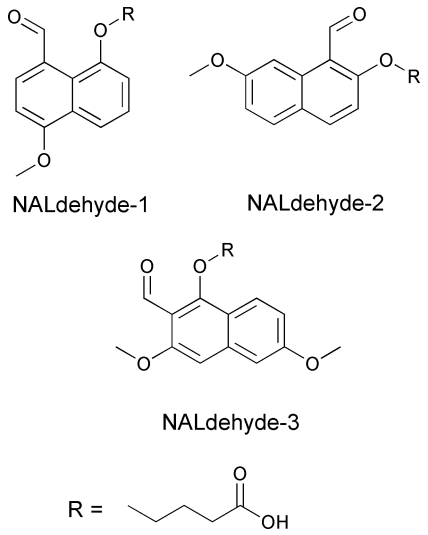


Fig. 2 Structure of two recently reported dialkoxynaphthaldehydes (NALdehyde-1 and NALdehyde-2) and the new trialkoxynaphthaldehyde (NALdehyde-3).

Relatively few highly substituted naphthalenes are known. The classical approach to aromatic compounds is electrophilic aromatic substitution, but in the case of naphthalene (and polycyclic aromatic compounds in general), this gives rise to problems with the regiochemistry due to a much larger number of possible isomers. Another problem is that many potential starting materials of the polycyclic aromatic hydrocarbon type with a suitable substitution pattern are no longer commercially available.[15]

Substituted naphthalenes with the type of substitution pattern needed in this study are furthermore difficult to synthesize by classical methods due to the non-symmetrical nature of the target. Many hydroxy substituted naphthalene compounds have been synthesized by an alkali melting procedure *via* the corresponding sulfonic acid derivative. This procedure is somewhat harsh, and it does not introduce the desired functionalities on the aromatic core. When dealing with highly substituted naphthalene substrates one also has to take into account the so called *peri*-effect, defined as the non-bonding repulsive interaction between substituents in the 1- and the 8-positions on the naphthalene core.[16] The above considerations led us to look into strategies based on building the naphthalene derivative from a suitable benzene derivative. Several methods for synthesis of naphthalenes from benzene derivatives exist and this topic has been recently reviewed.[17] In the development of the present procedure, scalability and ease was important, and this was achieved by combining the reported procedure on naphthoresorcinol with improvements disclosed in the recent literature on acid-catalyzed ring-closing reactions with naphthalene systems.[18,19]

The design of the three different NAL handles incorporates distinct features. Common for NALdehyde-1 and NALdehyde-2 is that they are dialkoxynaphthaldehydes. In NALdehyde-1 one of the alkoxy-groups is placed in the *peri*-position, enabling investigation of the effect of the closer proximity of the alkoxy group [the 1–8 distance (*peri*-distance) in naphthalenes is shorter than the 1–2 distance (*ortho*-distance)] on stability of the carbenium ion formed during the cleavage of the products from the solid phase.[16] NALdehyde-2 has a methoxy group in the *ortho* position to the aldehyde, thus giving a more common *ortho* stabilizing effect on the stability of the carbenium ion as used in the benzene derived BAL handles.[7] NALdehyde-3 is a trialkoxynaphthaldehyde where the methoxy substituents are placed such that it becomes an extended equivalent to the trialkoxybenzaldehyde based BAL handles. Thus we anticipated that NALdehyde-3 would produce a more acid-sensitive handle than NALdehyde-1 and NALdehyde-2. Furthermore, NALdehyde-3 has the aldehyde functionality in the 2-position on the naphthalene core as compared to the 1-position of the aldehyde in NALdehyde-1 and NALdehyde-2. This illustrates the additional possibilities that are present when extending the aromatic core from benzene to naphthalene.

Results and discussion

The synthesis of NALdehyde-3 (**10**) started from 3-methoxyphenyl acetic acid, as outlined in Scheme 1. Treatment of 3-methoxyphenylacetic acid with $SOCl_2$ in CH_2Cl_2 gave the acid chloride **2** that was conveniently reacted directly with the magnesium salt of diethyl malonate to produce the adduct **3**.[18] Treatment of **3** with methanesulfonic acid at rt yielded the ring-closed naphthalene compound **4** in a highly regioselective manner. This selectivity followed the general trend for this type of cyclisation, and was anticipated due to steric reasons.[19] The regiochemical outcome of the reaction was confirmed by X-ray structure elucidation of derivatives (*vide infra*) and only minor amounts (less than 1%) of the unwanted regioisomer was observed. Methylation of **4** by treatment with excess $(CH_3O)_2SO_2$ in dry acetone yielded **5** in excellent yield. The ester group in **5** was reduced to the alcohol **6** with $LiAlH_4$ in ether and subsequently oxidized to the aldehyde **7** with PDC in 84% yield. X-ray structure elucidation confirmed the structure of **7** (Fig. 3).‖ Selective mono-demethylation in the 1-position of **7** was achieved using BBr_3 in CH_2Cl_2 at 0 °C yielding **8** in 86% yield as a yellow crystalline material. Performing the reaction using the same reaction conditions at −78 °C did not change either the yield or the selectivity. A small amount of demethylation (approximately 1%) was observed in the 3-position yielding naphthol **11** (Scheme 2). The regiochemistry of the two different naphthols was confirmed by single crystal X-ray crystallography (Fig. 4) and 2D NMR spectroscopy (see crystal data). Attachment of the valeric acid spacer, thus completing the synthesis of the handle, was achieved by reacting the naphthol compound **8** with ethyl 5-bromovalerate in DMF with K_2CO_3 as the base, followed by hydrolysis of the ethyl ester with dilute NaOH in THF to yield **10** as the target handle, NALdehyde-3.

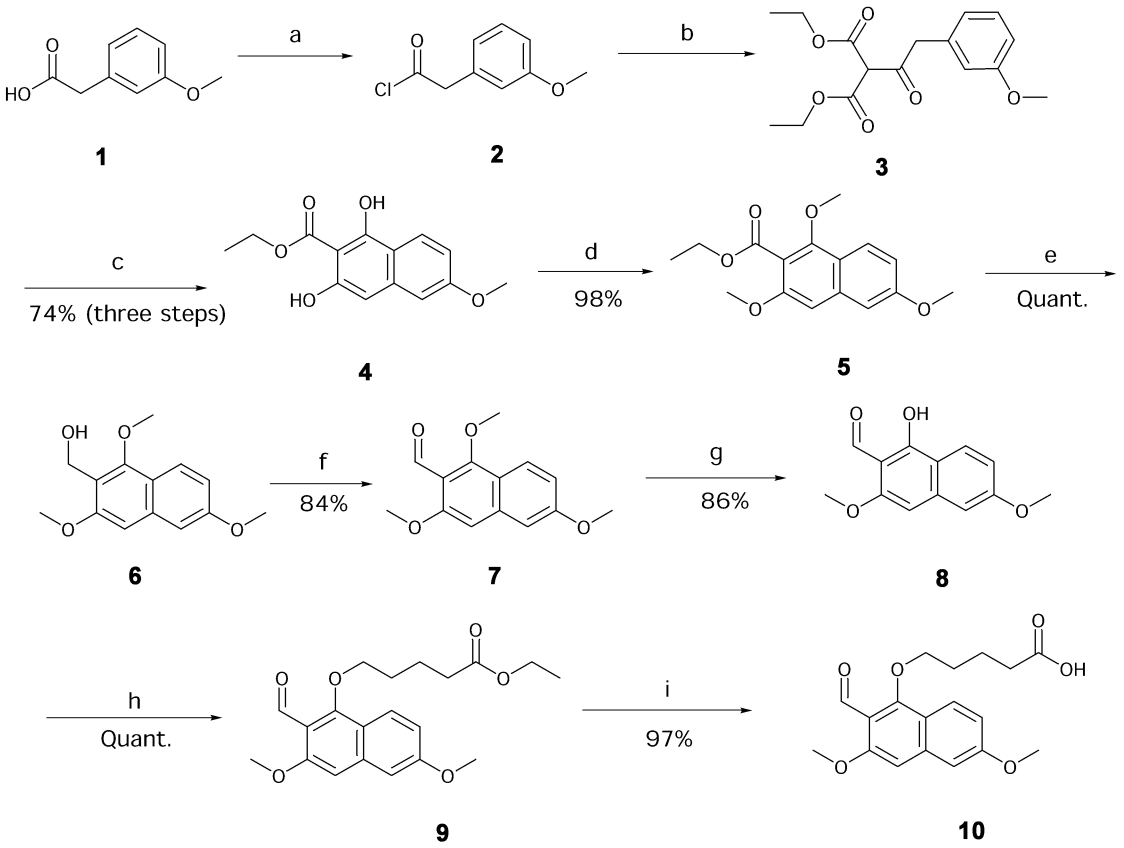
The high regioselectivity of the demethylation step from **7** to **8** was surprising. Methoxy groups placed in the *ortho*-position to an aldehyde functionality, are known to increase reactivity towards demethylation due to chelation control.[20,21] Thus, we expected the demethylation to take place in the 1- and 3-positions of the naphthalene without pronounced selectivity. The surprising selectivity led us to examine the crystal structure of the reactant **7** and the main product **8**. The crystal structure of the 1,3,6-trimethoxynaphthalene-2-carbaldehyde (**7**) shows interesting features (Fig. 3).

The methyl group of the 1-methoxy group is locked in a position that is 76.9° out of plane with the aromatic core, and the aldehyde oxygen is locked in a position pointing towards the 1-methoxy group. Also, the C–C bond connecting the aldehyde

¶ In naming the new naphthaldehyde 'NALdehyde-3' we follow the precedent set by the name 'PALdehyde'; see ref. 5.

‖X-Ray data for **7**. $C_{14}H_{14}O_4$, $M = 246.25$, monoclinic system, space group P_{21}/c, $a = 3.9760(3)$, $b = 11.8250(10)$, $c = 24.3230(16)$ Å, $\beta = 95.029(7)°$, $Z = 4$, $V = 1139.17(16)$ Å^3, $D_c = 1.436$ g cm^{-3}, μ(Mo Kα) = 0.105 cm^{-1}, crystal dimensions of 0.16 × 0.14 × 0.54 mm, $R_{init} = 0.1484$, final $R = 0.0548$ using 3941 independent reflections.

CCDC reference numbers 248480 and 248481. See http://www.rsc.org/suppdata/ob/b4/b412971g/ for crystallographic data in .cif format.



Scheme 1 Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2; (b) Mg, EtOH, $CH_2(CO_2Et)_2$, ether; (c) CH_3SO_3H, 74% (3 steps); (d) $(CH_3O)_2SO_2$, K_2CO_3, acetone, 98%; (e) $LiAlH_4$, ether, quant.; (f) PDC, CH_2Cl_2, 84%; (g) BBr_3, CH_2Cl_2, 0 °C, 86%; (h) ethyl 5-bromovalerate, K_2CO_3, DMF, 60 °C, quant.; (i) 2M NaOH (aq), THF, 97%.

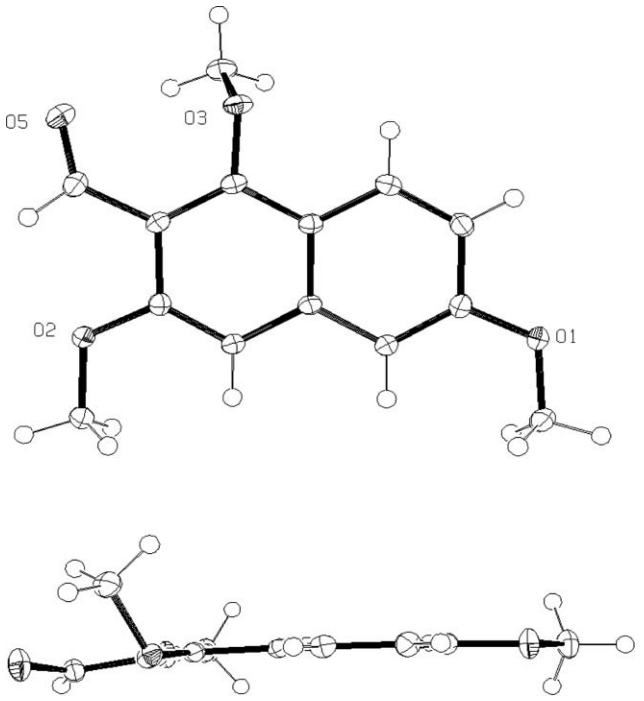
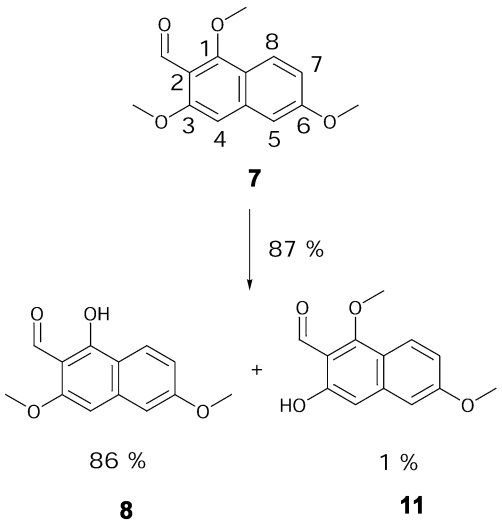


Fig. 3 Crystal structure of compound **7**, illustrating that the methyl moiety of the 1-methoxy group is twisted out of plane with the naphthalene ring. Also, the aldehyde moiety is twisted slightly out of the plane.

to the aromatic part of the molecule is out of plane with the aromatic core. The plane spanning C2, C3 and the aldehyde carbon is 7.4° out of the aromatic plane. These deviations from planarity are due to the sterically congested nature of the



Scheme 2 Reagents and conditions: BBr_3, CH_2Cl_2, 0 °C.

molecule, and they are an illustrative example of the effect of *peri*-substitution.[16] The protons in the 4- and 8-positions on the naphthalene core 'flank' the methoxy groups in the 3- and 1-positions, respectively, and these methoxy groups again 'flank' the aldehyde group in the 2-position. As a consequence, there is not enough space to place both the methoxy groups and the aldehyde group in the plane of the aromatic core. This forces the methyl moiety of the methoxy group in the 1-position out of the aromatic plane, because of steric repulsion (the *peri*-effect). This, in turn, forces the C–C bond to the aldehyde slightly out of the aromatic plane. In the demethylated product **8**, some of the steric strain has been relieved, as seen from the crystal structure where all substituents are in plane with the aromatic

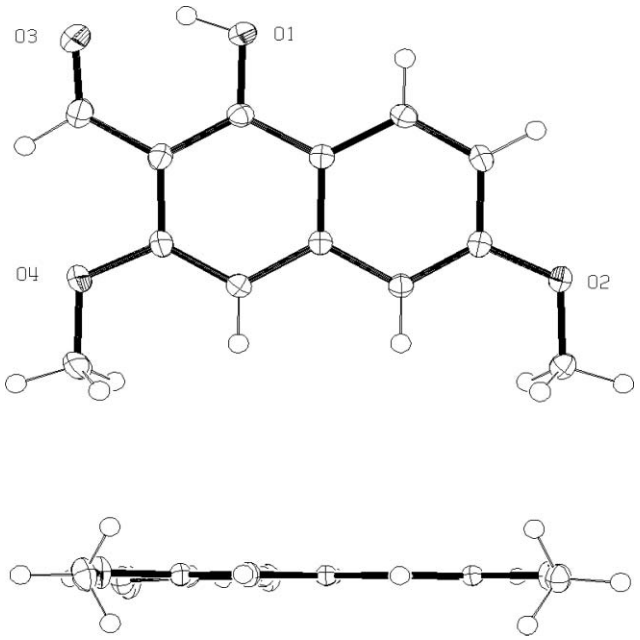


Fig. 4 Crystal structure of compound **8** illustrating the total planarity of the compound.

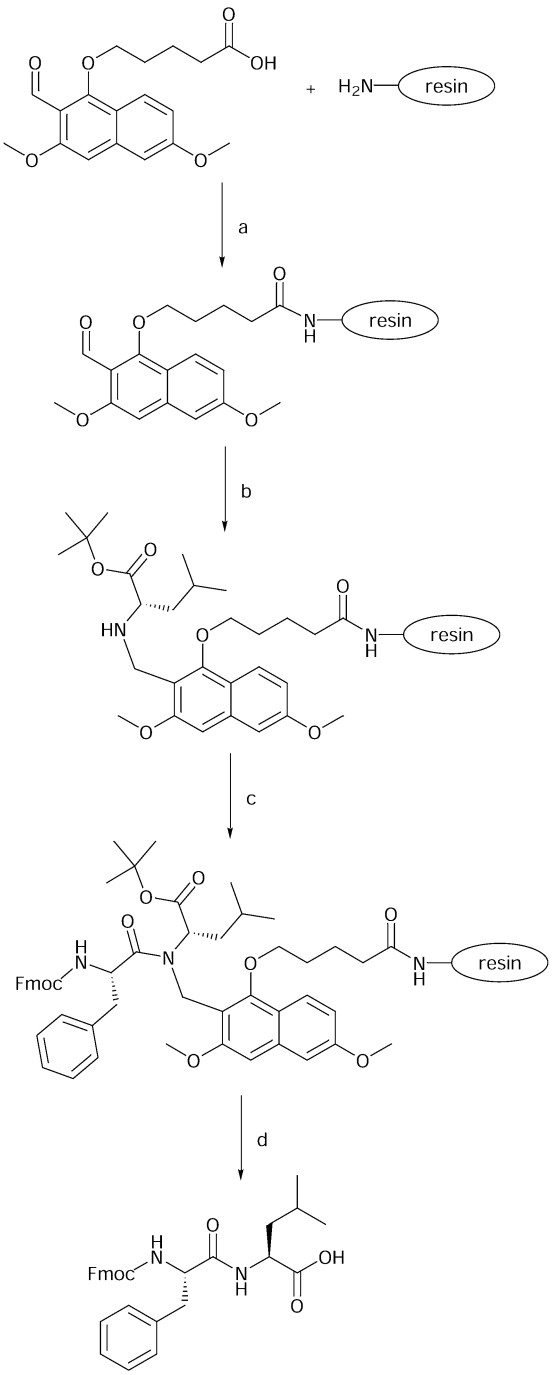
core (Fig. 4).‡‡ Thus, we propose steric relief as the driving force deciding the regioselectivity in the demethylation reaction, although increased basicity of O1 due to reduced overlap of the oxygen lone pairs with the aromatic ring could also play a role.

In order to evaluate NALdehyde-3 as a handle for solid-phase peptide synthesis, NALdehyde-3 (**10**) was anchored to a high loading aminomethylated poly(styrene) resin with *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU) in the presence of *N*,*N*-diisopropylethylamine (DIEA) in DMF. Unreacted free amine sites on the resin were capped with acetic acid anhydride.

Then a reductive amination of the free aldehyde on NAL-3 with H-Leu-OtBu·HCl and $NaBH_3CN$ in DMF was conducted twice, followed by acylation of the secondary amine functionality on the handle with the symmetric anhydride of Fmoc-Phe-OH (also conducted twice) resulting in a loading of 0.21 mmol g^{-1} (initial loading was 0.36 mmol g^{-1}). The solid-phase synthesis of the model dipeptide Fmoc-Phe-Leu-OH on the NAL-3 is outlined in Scheme 3.

To investigate the acid-lability of the NAL-3 handle, the synthesized dipeptide was cleaved from the solid support using different acidic conditions, as shown in Table 1. Using 'high acid' conditions (TFA–CH_2Cl_2 19 : 1) and long reaction times, the dipeptide was released in up to 56% yield (Scheme 3). Acidolytic release from the NAL-3 resin with TFA–CH_2Cl_2 (19 : 1) gave the dipeptide in 44% (16 h), 47% (24 h) and 56% (48 h) with high HPLC purity. The overall yield of released dipeptide was around 13% under high acid conditions for 1 h. Applying lower concentrations of TFA in CH_2Cl_2 or adding H_2O resulted in lower cleavage yields releasing only traces of the dipeptide after 1 h. At these 'low acid' conditions a relatively large amount of the fully protected peptide was released, however also in modest yield. Thus, to our surprise, the NAL-3 handle did not exhibit as high acid-lability as the dialkoxynaphthalene based handles previously reported.

The above mentioned cleavage yields were determined from the HPLC areas of released Fmoc-Phe-Leu-OH in comparison with a Fmoc-Phe-OH standard curve. Fmoc-Phe-Leu-OH was synthesized on a preparative scale using the NAL-3 handle and cleaved using 95% TFA in CH_2Cl_2 in 50% crude yield (see supplementary information for HPLC†).



Scheme 3 Solid-phase synthesis of Fmoc-Phe-Leu-OH on NAL-3. Reagents and conditions: (a) HBTU–DIPEA–HOBt–DMF, 16 h, rt, then 30% Ac_2O, DMF–DIPEA (catalytic amount), 3 h, rt; (b) H-Leu-OtBu·HCl–$NaBH_3CN$–DMF, 16 h, rt; (c) Fmoc-Phe-OH–DIPCDI, CH_2Cl_2–DMF 9 : 1, 16 h, rt; (d) acidolytic release from NAL-3 resin, see Table 1.

We had anticipated that NAL-3 would be highly acid-labile, and more so than the naphthalene based handles with fewer alkoxy substituents (NAL-1 and NAL-2). Also, the differences between NAL-3 *vs.* NAL-1 and NAL-2 were expected to parallel the differences between the BAL handles based on dialkoxybenzaldehyde and trialkoxybenzaldehyde.[22] The crystal structure of the intermediate aldehyde (**7**) clearly showed that the methoxy group in the *peri*-position and the aldehyde group are twisted out of the aromatic plane. Co-planarity is of paramount

‡‡ X-Ray data for **8**. $C_{13}H_{12}O_4$, $M = 232.23$, monoclinic system, space group P_{21}/c, $a = 18.985(2)$, $b = 3.9180(6)$, $c = 14.404(3)$ Å, $\beta = 103.443(11)°$, $Z = 4$, $V = 1049.3(3)$ Å^3, $D_c = 1.470$ g cm^{-3}, μ(Mo Kα) = 0.109 cm^{-1}, crystal dimensions of 0.06 × 0.12 × 0.61 mm, $R_{init} = 0.1449$, final $R = 0.0519$ using 3078 independent reflections.

Table 1 Cleavage yields using different acidic conditions

Cleavage conditions[a]	Time/h	Cleavage yield/%[b]
95% TFA–DCM	1	13
95% TFA–H_2O	1	3
50% TFA–DCM	1	9
50% TFA–2% TIS–DCM	1	6
5% TFA–DCM	1	<1
5% TFA–2% TIS–DCM	1	<1
1% TFA–DCM	1	<1
1% TFA–2% TIS–DCM	1	<1
95% TFA–DCM	16	44
95% TFA–DCM	24	47
95% TFA–DCM	48	56

[a] All cleavage experiments were conducted at rt. [b] Cleavage yields were calculated by comparison with a standard curve.

importance for achieving maximum resonance stabilization of the naphthylic carbenium formed during the cleavage process from the solid phase, and hence the reduced acid lability we observe. We suggest that steric hindrance (due to the *peri*-effect) forces the carbenium ion into the naphthyl methylene position that is formed during the cleavage process of the substrate from the solid support, out of plane with the aromatic core.

The naphthyl methylene carbon atom becomes sp^2 hybridized when the carbenium is formed. The p-orbital in the naphthyl methylene carbenium ion must have the maximum overlap with the π-orbitals from the aromatic core in order to gain the maximum carbenium stabilization. If planarity of the carbenium ion is lost, the carbenium ion stability and the acid-lability of the handle, are also reduced. A related type of behavior has recently been observed in highly substituted derivatives of 1,8-bis-(dimethylamino)naphthalene ('proton sponge').[23]

Conclusions

We have developed a convenient synthesis of a number of novel highly substituted naphthalene compounds and a facile synthetic route to the first backbone amide linker based on a trialkoxynaphthaldehyde. The synthesis of novel NALdehyde-3 (**10**) features a selective ring-closing reaction to form the naphthalene system **4** and a highly selective demethylation reaction to form the naphthol compound (**8**). Using standard procedures for Fmoc-based solid phase synthesis a dipeptide was prepared on the new NAL-3 handle. The final products were cleaved with high crude purity using TFA in CH_2Cl_2 (19 : 1).

Experimental

High-loading aminomethylated PS resin, all amino acids, and HBTU were obtained from NovaBioChem, HOBt from Quantum Richelieu. Solid-phase reactions were performed in poly(propylene) syringes equipped with a poly(ethylene) filter, placed on a shaker. HPLC-MS analysis was performed on a Shimadzu 2010, using a Phenomenex Jupiter C5 column (5 μ, 300 Å). Gradient: Linear 1 mL min^{-1} from 3% to 95% buffer B over 18 min (buffer A: 0.025% TFA in H_2O; buffer B: 0.025% TFA in 90% aq. CH_3CN). UV analysis was performed at a Perkin Elmer Lambda 2 UV-vis spectrometer. Solvents were HPLC grade and were used as received. ^1H NMR and ^{13}C NMR spectra were recorded on a 300 MHz NMR (Varian or Bruker Avance) instrument (300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR) or on a 400 MHz NMR (Bruker) instrument (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR). Proton chemical shifts are reported in ppm downfield from tetramethylsilane (TMS) and carbon chemical shifts in ppm downfield of TMS using the resonance of the deuterated solvent as internal standard. Assignments of ^1H NMR signals were based on HSQC, HMBC, NOESY, and COSY spectra. Melting points were measured on a Büchi B-140 apparatus and are uncorrected. Elemental analysis was performed by Mrs Karin Linthoe. Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol JMS-HX 110A Tandem Mass Spectrometer in the positive ion mode using m-NBA as the matrix. HRMS were recorded on a Micromass Q-TOF apparatus using electrospray ionisation (ESI) technique. All column chromatography was performed on Merck Kieselgel 60 (0.015–0.040 mm) using the DCVC technique.[24]

2-[2-(3-Methoxyphenyl)-acetyl]-malonic acid diethyl ester (3)

(3-Methoxyphenyl)-acetic acid **1** (30.0 g, 180.5 mmol) was dissolved in CH_2Cl_2 (200 mL) and $SOCl_2$ (25.8 g, 15.8 mL, 217 mmol) was added in one portion. The reaction mixture was heated to reflux for 2 h until no more HCl gas evolved. The reaction mixture was evaporated to dryness *in vacuo* to yield the crude acid chloride **2** (33.4 g *ca.* 100%). This was sufficiently pure for further use. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.23–7.32 (m, 1H), 6.81–6.91 (m, 3H), 4.12 (s, 2H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.2, 132.8, 130.2, 122.0, 121.9, 115.5, 113.8, 55.5, 52.3; *m/z* (GC-MS) 182 (M^+).

In a three-necked round-bottomed flask (500 mL) equipped with a dropping funnel and a reflux condenser, Mg turnings (4.13 g, 175 mmol), dry CCl_4 (0.3 mL) and freshly distilled diethylmalonate (13.61 g, 12.90 mL, 85.0 mmol) were placed under a N_2 atmosphere. Anhydrous EtOH (30 mL) was added. When the reaction started to subside, additional diethylmalonate (13.61 g, 12.90 mL, 85.0 mmol) was added and the reaction proceeded to completion. The reaction mixture was cooled to rt, dry diethyl ether (60 mL) was added and the mixture was heated to reflux for 60 min. Then 3-methoxyphenylacetyl chloride **2** (33 g, 178.8 mol) in dry ether (90 mL) was added over a 30 min period. After the addition, the reaction mixture was heated to reflux for 20 min. The reaction mixture was cooled to rt and water (30 mL) was added dropwise over a period of 10 min. The organic phase was washed with water (2 × 30 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield an off-white semi-solid material. TLC (EtOAc–heptane 1 : 1, R_f *ca.* 0.6) showed a high purity. Crude yield 53.0 g, 100%. The crude product was sufficiently pure for further synthesis. ^1H NMR ($CDCl_3$, 300 MHz) δ 13.25 (enol form of α-H, br s, 5% of 3H), 6.80–6.90 (m, 2H), 6.45–6.60 (m, 2H), 3.98 (α-H, br s, 95% of 3H), 3.45–3.60 (m, 7H), 1.05–1.20 (m, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.1, 138.6, 129.4, 128.8, 121.5, 114.7, 113.7, 111.4, 61.7, 60.4, 54.8, 44.4, 13.9; *m/z* (FABMS) 639 ($2M + Na^+$).

1,3-Dihydroxy-6-methoxy-naphthalene-2-carboxylic acid ethyl ester (4)

Anhydrous CH_3SO_3H (100 mL) was added to the malonic ester derivative **3** (32.5 g, 3.24 mmol) without cooling and the ester dissolved by gentle heating with a heat gun. The reaction mixture was stirred overnight at rt giving a reddish inhomogeneous slurry. The reaction mixture was poured into ice water (2 L) resulting in precipitation of a pale yellow solid. The mixture was extracted with CH_2Cl_2 (2 × 1 L) and the combined organic extracts were dried and evaporated to dryness *in vacuo* yielding a pale yellow solid material. This was purified by dry column vacuum chromatography (from heptane to EtOAc–heptane 1 : 1 with 5% increments). Yield 20.46 g, 74% as an off-white solid; mp 112–113 °C (EtOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 11.15 (br s, 1H; O*H*), 9.05 (br s, 1H; O*H*), 8.13 (d, 1H, J = 9.2 Hz; H8), 6.91 (dd, 1H, J = 9.2, 2.5 Hz; H7), 6.83 (d, 1H, J = 2.5 Hz; H6), 6.67 (s, 1H; H4), 4.60 (q, 2H, J = 7.1 Hz, CH_2), 3.91 (s, 3H, OCH_3), 1.52 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.0, 161.4, 139.9, 125.9, 115.5, 115.4, 114.4, 104.2, 104.1, 101.6, 95.6, 62.6, 55.2, 14.3; *m/z* (FABMS) 263.1 ($M + H^+$); Anal. calcd. for $C_{14}H_{14}O_5$: C, 64.12; H, 5.38. Found: C, 63.89; H, 5.21.

1,3,6-Trimethoxynaphthalene-2-carboxylic acid ethyl ester (5)

Diol **4** (5.52 g, 21.05 mmol) was dissolved in dry acetone (150 mL) and K_2CO_3 (9.67 g, 69.5 mmol) was added followed by addition of dimethylsulfate (46.64 mmol, 2.2 eq). The mixture was stirred at rt for 48 h. Water (200 mL) was added and the mixture extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic extracts dried (Na_2SO_4) and evaporated to dryness *in vacuo*. Recrystallisation from EtOH yielded compound **5** as colorless needles. Yield 5.97 g, 98%; mp 101–102 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.86 (d, 1H), 6.94–6.97 (m, 2H), 6.78 (s, 1H), 4.37 (q, 3H), 3.93 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 1.34 (t, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.4, 159.1, 154.9, 154.6, 137.0, 124.2, 118.0, 116.3, 115.2, 105.5, 101.1, 63.0, 61.4, 55.8, 55.2, 14.2; *m/z* (FABMS) 290.1 (M^+); Anal. calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found: C, 66.53; H, 6.20.

(1,3,6-Trimethoxynaphthalen-2-yl)-methanol (6)

Ester **5** (5.97 g, 20.56 mmol) was dissolved in dry ether (300 mL) and added to an ice-cold stirring solution of $LiAlH_4$ (2.34 g, 61.7 mmol) in dry ether (100 mL) over a 5 min period under an N_2 atmosphere. This mixture was stirred at rt for 4 h. Water was added until the mixture became turbid (*ca.* 8 mL) and the mixture was filtered through a plug of $MgSO_4$. The $MgSO_4$ was washed with additional ether and the combined ethereal extracts were evaporated to dryness *in vacuo*. This yielded the alcohol as an off-white solid. Yield 5.10 g, 100%; mp 78–80 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.93 (d, 1H, J = 9.0 Hz), 7.01–7.07 (m, 2H), 6.89 (s, 1H), 4.89 (s, 2H), 3.98 (s, 3H), 3.96 (s, 3H), 3.91 (s, 3H), 2.53 (br s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 158.8, 157.2, 155.1, 136.1, 124.0, 119.3, 118.5, 116.0, 105.5, 101.1, 63.4, 55.7, 55.5, 51.1; *m/z* (FABMS) 248.1 (M^+); Anal. calcd.. for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.75; H, 6.55.

1,3,6-Trimethoxy-naphthalene-2-carbaldehyde (7)

Alcohol **6** (3.00 g, 12.08 mmol) was dissolved in CH_2Cl_2 (120 mL) and PDC (9.12 g, 24.16 mmol) was added in one portion. The reaction mixture was stirred under N_2 overnight at rt. The reaction mixture was filtered through a plug of celite, and the organic phase was washed with water (100 mL), evaporated to dryness *in vacuo* and purified by dry column vacuum chromatography (heptane to heptane–EtOAc 1 : 1 with 5% increments). Yield: 2.50 g, 84% as a white solid; mp 111–112 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.57 (s, 1H; C*H*O), 8.06 (d, 1H, J = 8.7Hz; H8), 7.05 (dd, 1H, J = 8.7, 1.7 Hz; H7), 7.02 (d, J = 1.7 Hz; H5), 6.86 (s, 1H; H4), 4.04 (s, 3H; CH_3O1), 3.98 (s, 3H; CH_3O), 3.93 (s, 3H; CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.1, 161.4, 158.4, 139.8, 125.7, 118.4, 117.0, 115.6, 105.2, 101.3, 64.3, 55.8, 55.3; *m/z* (FABMS) 247.1 (M + H^+); Anal. calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 67.87; H, 5.71.

1-Hydroxy-3,6-dimethoxynaphthalene-2-carbaldehyde (8)

Trimethoxy aldehyde **7** (1.00 g, 4.06 mmol) was dissolved in CH_2Cl_2 (140 mL) and cooled to 0 °C under N_2. Then BBr_3 in CH_2Cl_2 (0.406 mL, 1 M, 4.06 mmol) was added dropwise over a period of 30 min resulting in a deep red color. The reaction mixture was allowed to reach rt over a period of 30 min. Water (150 mL) was added slowly to the vigorously stirred solution and the phases were separated. The aqueous phase was extracted with additional CH_2Cl_2 (2 × 100 mL) and the combined organic extracts were evaporated to dryness *in vacuo* and purified by dry column vacuum chromatography (heptane to EtOAc with 5% increments) to yield two products; title product **8** (815 mg, 86%) and the regioisomer **11** (15 mg, 1%). **8**: mp 111–112 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 13.59 (s, 1H; O*H*), 10.18 (s, 1H; C*H*O), 8.08 (d, 1H, J = 8.7 Hz; H8), 6.87 (dd, 1H, J = 9.2, 2.3 Hz; H7), 6.82 (broad s, 1H; H5), 6.33 (s, 1H; H4), 3.86 (s, 3H; CH_3O), 3.83 (s, 3H, CH_3O); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 193.5, 164.1, 161.9, 157.5, 140.9, 126.3, 115.4, 114.9, 106.7, 105.8, 95.4, 55.4, 55.3; *m/z* (FABMS) 233.0 (M + H^+); Anal. calcd. for $C_{13}H_{12}O_4$: C, 67.23; H, 5.21. Found: C, 67.08; H, 5.06.; **11**: mp 98–99 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.96 (s, 1H; O*H*), 10.35 (s, 1H; C*H*O), 7.89 (d, 1H, J = 9.2 Hz; H8), 6.91 (dd, 1H; J = 9.2, 2.5 Hz; H7), 6.84 (d, 1H, J = 2.6; H5), 6.83 (s, 1H; H4), 4.01 (s, 3H; CH_3O1), 3.81 (s, 3H; CH_3O6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.0, 164.0, 161.5, 157.3, 142.1, 125.0, 117.4, 112.5, 106.5, 104.6, 95.3, 66.0, 55.3; *m/z* (FABMS) 233.0 (M + H^+); Anal. calcd. for $C_{13}H_{12}O_4$: C, 67.23; H, 5.21. Found: C, 67.02; H, 5.28.

5-(2-Formyl-3,6-dimethoxynaphthalen-1-yloxy)-pentanoic acid ethyl ester (9)

Naphthol **8** (104 mg, 0.448 mmol) was dissolved in anhydrous DMF (7 mL) and K_2CO_3 (75 mg, 0.54 mmol) was added. Ethyl 5-bromovalerate (112 mg, 0.54 mmol) was added and the reaction mixture was stirred overnight at 60 °C and then allowed to reach rt. Water (15 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were evaporated to dryness (oil pump, 2 mm Hg) and purified by dry column vacuum chromatography to yield a yellow oil. Yield 0.157 g, 97%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.54 (s, 1H), 8.02 (d, 1H), 6.99–7.03 (m, 2H), 6.84 (s, 3H), 4.14 (q, 2H, J = 7.14 Hz), 4.10 (t, 2H, J = 6.04 Hz, 2H), 3.97 (s, 3H), 3.92 (s, 3H), 2.42 (t, 2H, J = 6.59 Hz), 1.84–2.02 (m, 4H), 1.26 (t, 3H, J = 7.14 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 189.0, 173.2, 160.8, 160.5, 158.3, 139.7, 125.6, 118.6, 116.9, 115.7, 105.2, 101.2, 76.9, 60.2, 55.8, 55.3, 33.9, 29.6, 21.5, 14.1; *m/z* (FABMS) 361.11 (M + H^+); Anal. calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.28; H, 6.75.

5-(2-Formyl-3,6-dimethoxynaphthalen-1-yloxy)-pentanoic acid (10, NALdehyde-3)

Ester **9** (762 mg, 2.11 mmol) was dissolved in THF (20 mL) and dilute NaOH (1 M, 10 mL) was added. The reaction mixture was stirred overnight at rt. The reaction mixture was acidified by addition of 2 M aq. HCl (20 mL) resulting in a green solution. This was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic extracts were evaporated to dryness *in vacuo*. The residue was dissolved in EtOH (96%, 30 mL), decolourised with charcoal and filtered through a plug of celite. Evaporation of the EtOH resulted in a bright yellow oil which was precipitated as a fine yellow powder by stirring with ether at 0 °C. Yield 0.681 g, 97%; mp 95–97 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.56 (s, 1H), 8.04 (d, 1H, J = 9.0 Hz; H8), 7.04 (dd, 1H, J = 9.0, 2.5 Hz; H7), 7.01 (d, 1H, J = 2.3 Hz; H5), 6.86 (s, 1H; H4), 4.12 (t, 2H, J = 5.9 Hz), 3.98 (s, 3H; CH_3O), 3.94 (s, 3H, CH_3O), 2.52 (t, 2H, J = 6.5 Hz; H2′), 1.90–2.04 (m, 4H; H3′, H4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.2, 178.9, 160.8, 160.4, 158.3, 139.7, 125.7, 118.6, 116.9, 105.2, 101.2, 76.8, 55.8, 55.3, 33.6, 29.5, 21.2; *m/z* (FABMS) 331.1 (M − H^-); Anal. calcd. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 64.83; H, 6.32.

Solid-phase anchoring of NALdehyde-3

NALdehyde-3 (**10**) (0.28 g, 0.82 mmol) and HBTU (0.30 g, 0.80 mmol) were dissolved in DMF (5 mL), DIEA (0.28 mL, 1.64 mmol) was added and the mixture was gently shaken for 5 min at rt. The clear solution was added to aminomethyl-poly(styrene) resin (0.57 g, 0.21 mmol, loading 0.36 mmol g^{-1}) and the suspension was shaken for 16 h at rt. The resin was washed with DMF (10 times). Residual free amino groups on the resin were capped using 30% acetic anhydride in DMF (5 mL) together with a catalytic amount of DIEA followed by shaking for 2 h at rt. Finally, the resin was washed with DMF (10 times) and CH_2Cl_2 (10 times), shrunk with methanol and air dried.

Solid-phase synthesis of Fmoc-Phe-Leu-OH

NALdehyde-3 derivatised resin (0.30g, 0.11 mmol, theoretical load 0.36 mmol g^{-1}) was swollen in DMF (2 mL), followed by addition of HLeu-O*t*Bu·HCl (0.24 g, 1.08 mmol) and $NaBH_3CN$ (67 mg, 1.08 mmol). The mixture was shaken for 16 h at rt. The resin was washed once with DMF and the procedure was repeated. Hereafter, the resin was washed with DMF (10 times) and CH_2Cl_2 (10 times). The resin was swollen in a CH_2Cl_2–DMF 9 : 1 mixture (5 mL), Fmoc-Phe-OH (0.42 g, 1.08 mmol) and DIPCDI (85 μL, 0.54 mmol) were added and the mixture was shaken for 16 h at rt creating a viscous suspension. The resin was washed with DMF (10 times) and the procedure was repeated. Hereafter, the peptidyl-resin was washed with DMF (10 times) and CH_2Cl_2 (10 times), shrunk with methanol and air dried. To release the dipeptide, the peptidyl-resin (0.100 g) was put in a filter syringe and treated with TFA-CH_2Cl_2 (19 : 1) for 1 h, the solvents were removed *in vacuo* and the remaining material was triturated with diethyl ether. Yield (crude): 6 mg (50%). HPLC-MS: t_R: 24.3 min; *m/z* (ESI) 501 ($M + H^+$), 523 ($M + Na^+$).

Determination of resin substitution using the Fmoc-group

Three portions of resin (approximately 5 mg, 2 μmol theoretical amount of peptide) were suspended in a 20% piperidine–DMF solution (25 mL). The mixtures were shaken for 30 min, and the absorbance (290 nm) was measured using piperidine–DMF (1 : 4) as blank scan reference. The loading of the peptidyl-resin was measured to 0.21 mmol g^{-1}.

Cleavage studies

Portions of peptidyl-resin (approximately 5 mg) were placed in filter syringes and subjected to acidolytic cleavage for the time stated in Table 1 by 0.5 mL of a cleavage mixture as stated in Table 1. After removal of the solvents *in vacuo* the remaining material was dissolved in acetonitrile–H_2O (1 : 1, 0.5 mL) and analysed by HPLC-MS at 220 nm. The cleavage yields were determined from the HPLC areas of released Fmoc-Phe-Leu-OH in comparison with a Fmoc-Phe-OH standard curve having an initial concentration of 0.25 mg mL^{-1} Fmoc-Phe-OH in acetonitrile–H_2O (1 : 1). The results are shown in Table 1.

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