One-step labelling of sphingolipids *via* a scrambling cross-metathesis reaction†

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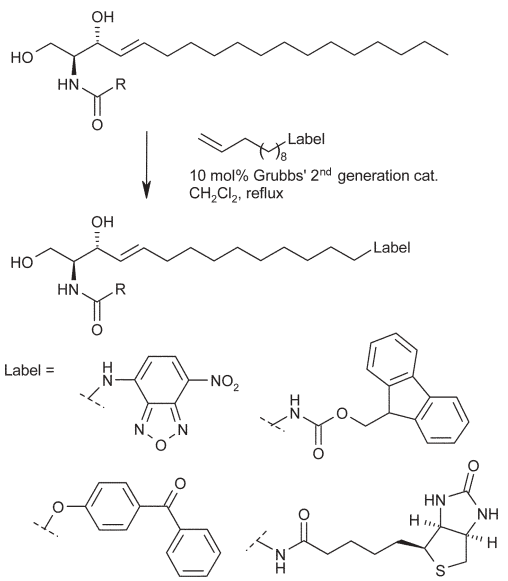
The alkyl chain in the backbone of sphingosine derivatives can be exchanged with functionalised (labelled) side chains in a single step under cross-metathesis reaction conditions.

The discovery that the biological function of sphingolipids is not merely structural, but that most of these molecules act as extra- and intracellular signalling mediators has led to a tremendous interest in this area over recent years.[1] This development has stimulated the generation of tool compounds that can help solve biological problems. In particular, bioactive derivatives with an attached label or tag are useful for localisation, binding and metabolism studies.

Recently, our group developed the first cell-free, non-radioactive sphingosine kinase assay using a fluorescently labelled sphingosine analogue.[2] This compound was synthesised in nine steps following a linear route starting from (*S*)-Garner aldehyde.[3] In order to extend the labelling to other sphingolipids (such as ceramides and sphingomyelin) and to allow for the introduction of different labels, we searched for a more efficient and flexible approach.

Specifically, we envisaged that sphingosine derivatives with modifications in their alkyl chain might be accessible *via* a scrambling cross-metathesis reaction between the natural sphingolipid itself and a terminal olefin containing the label (Scheme 1).[4,5]



Scheme 1 Synthesis of labelled ceramides (Table 1, entries 1-9).

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† Dedicated to Prof. Steven V. Ley on the occasion of his 60th birthday.

Thus, we reacted commercially available *N*-octanoyl-D-erythro-sphingosine (C8-ceramide) with 4 equivalents of Fmoc-protected 10-undecenylamine and 10 mol% of 2nd generation Grubbs' catalyst[6] under standard conditions[7] (Table 1, entry 1). We observed gradual consumption of the ceramide starting material and formation of a slightly more polar product, in addition to homodimer formation of the terminal olefin. After 2.5 h the reaction was stopped and the product mixture was subject to chromatography on silica gel. The polar product isolated in 49% yield was proven to be the desired terminally substituted C8-ceramide. Moreover, the *E*-isomer was formed exclusively (*E*/*Z* > 95/5) as determined by ^{1}H NMR spectroscopy. As expected, the reaction of C8-ceramide with unprotected 10-undecenylamine did not yield any product (entry 2) due to catalyst scavenging by the basic amine functionality. We nevertheless investigated *N*-(10-undecenyl)-7-nitro-4-benzofurazan-amine[8] as the olefin component in the cross metathesis reaction, reasoning that the basicity and nucleophilic character of the amino functionality in this molecule is significantly reduced compared to an aliphatic amine (entry 3). Analogous to entry 1, a slightly more polar fluorescent product was formed, which was isolated and proven to be the desired NBD-labelled C8-ceramide. The way was now successfully paved for the synthesis of ceramides with a fluorescent label in the sphingosine backbone in one step from the parent compounds by the demonstrated compatibility of the amino-NBD functionality with the reaction conditions.

To investigate the scope of the one-step labelling procedure, we used various *N*-acylated sphingosine derivatives and other labels attached to the undecenyl residue (entries 4–9). As summarised in Table 1, all reactions gave the desired labelled products. In general, the yields of isolated products were moderate to good, except for entries 7–9 (for entries 8 and 9 the sulfur-containing biotin moiety

Table 1 Backbone-functionalised sphingosine derivatives by cross-metathesis reaction[7]

Entry	R	Label	Yield[a] (%)
1	$-C_7H_{15}$	-NH-Fmoc	49
2	$-C_7H_{15}$	$-NH_2$	0
3	$-C_7H_{15}$	-NH-NBD[b]	52
4	$-C_{15}H_{31}$	-NH-NBD	52
5	$-CH_3$	-NH-NBD	48
6	$-OC(CH_3)_3$	-NH-NBD	71
7	$-C_7H_{15}$	-O-BzPh[c]	20
8	$-CH_3$	-NH-biotin	22
9	$-C_7H_{15}$	-NH-biotin	14
10	Sphingosine	-NH-NBD	0
11	*N,N*-Dimethyl sphingosine·HCl	-NH-NBD	39
12	Sphingomyelin	-NH-NBD	5

[a] Isolated yields, not optimised; [b] NBD = 7-nitrobenzofurazan-4-yl; [c] BzPh = p-benzophenone.

might poison the catalyst to some extent). The major by-product in these reactions was always the homodimer of the terminal alkene. In one case, we also isolated the homodimer of the ceramide head group in 5% yield (entry 8).

Considering that (i) the products themselves are substrates for the catalyst, (ii) all reactions were performed under standard conditions[7] without any optimisation, and (iii) in most cases some amount of product was lost during extensive chromatography (silica gel, sephadex LH20, or prep-HPLC) on small scale to separate starting material, homodimers and catalyst, we deem this procedure highly efficient for the synthesis of backbone-labelled sphingosine derivatives. Besides the fast and easy access to valuable tool compounds in good yields, intriguing features of our procedure are the universally observed high *E*-selectivity of the newly formed double bond and the high compatibility with various functional groups. This is not only illustrated by the introduction of various labels but also of protected functionalities, for example, after Fmoc deprotection, the terminal amino group in the backbone (entry 1) can be used for further modification. A structurally related head group protected ω-aminosphingosine was previously synthesised in nine steps from commercially available materials.[9]

We then investigated additional selected sphingolipids as substrates. In analogy to entry 2 where the terminal olefin featured a primary amino function, sphingosine itself did not react with *N*-(10-undecenyl)-7-nitro-4-benzofurazanamine under standard conditions (entry 10). We did not investigate using sphingosine as a substrate for the cross-metathesis reaction any further, because entry into the sphingosine series is easily achieved by carbamate hydrolysis of the *N*-Boc-sphingosine series. Thus, acid treatment (HCl/MeOH or TFA) of the product resulting from entry 6 yielded the NBD-labelled sphingosine derivative quantitatively.[10] However, we were interested in preparing labelled *N,N*-dimethylsphingosine, which is a commonly used inhibitor of sphingosine kinases.[11] We hoped that a trialkylamine might behave differently to a primary amine and reacted the compound as the free base and as the hydrochloride salt with *N*-(10-undecenyl)-7-nitro-4-benzofurazanamine under standard conditions. The hydrochloride salt of *N,N*-dimethylsphingosine was successfully transformed into the NBD-labelled derivative (entry 11, Fig. 1), whereas the reaction with the free base was not as clean. Finally, we subjected the commercially available egg sphingomyelin (84% palmitoyl sphingomyelin: Avanti Polar Lipids # 860061) to this reaction and obtained pure NBD-backbone-labelled C16-sphingomyelin (Fig. 1) in one step following exhaustive silica gel and reversed phase chromatography. Several factors that need further investigation can attribute to the low yield of 5%, but the easy access to the starting material in gram amounts and the simplicity of our method makes it superior to any other synthetic procedure for the preparation of this product.

In summary, we have discovered a highly efficient method to introduce various functionalities into the backbone of sphingolipids in one step using the cross-metathesis reaction. This procedure provides an easy and flexible synthesis of labelled derivatives that are otherwise only accessible *via* laborious routes.

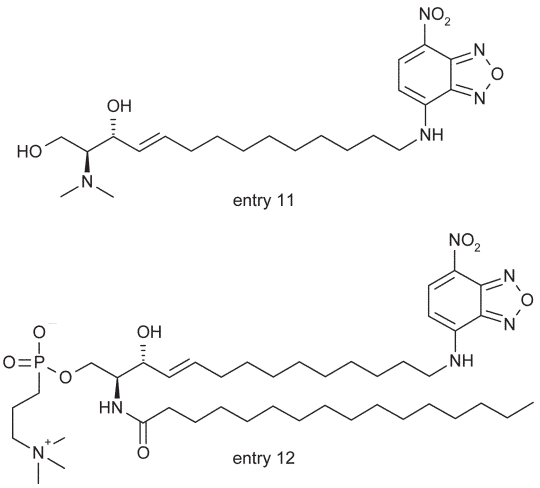

Fig. 1 NBD-backbone-labelled *N,N*-dimethylsphingosine and sphingomyelin (Table 1, entries 11 and 12).

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