Abstract

Fluorescence Resonance Energy Transfer (FRET) is the non-radiative transfer of energy from an excited fluorophore (or donor) to a suitable quencher (or acceptor) molecule. The energy transfer is influenced by the spectral overlap of the donor and acceptor molecules, their distance from each other and the relative orientation of the donor and acceptor transition dipoles.

The physical principle of FRET is used in a variety of applications including the measurement of protease activity of substrates, with the fluorophore separated from the quencher by a short peptide sequence containing the enzyme cleavage site. The spatial separation of the donor from the acceptor molecule by proteolysis of the peptide bond results in an increase in fluorescence because the energy transfer efficiency decreases exponentially with the separating distance.

In this monograph Bachem presents a range of highly sensitive FRET protease substrates for a variety of enzymes.
Fluorophores are substances which, like chromophores, absorb light in the UV or visible range. In contrast to chromophores they re-emit part of the light as radiation. This process is called fluorescence and can be illustrated by the energy level diagram suggested by A. Jablonski. Absorption of light \( (h\nu_A) \) causes an electron to be promoted from its electronic ground state (designated as \( S_0 \)) to an excited state (usually \( S_1 \)). Every energy state has several vibrational energy levels \( 0, 1, 2 \) etc. During the lifetime of the excited state, i.e. the time elapsed between excitation of the molecule and emission of the photon (usually between 1-10 ns) part of the energy is lost by internal vibration. As a result the wavelength of the emitted light \( (h\nu_F) \) is always longer than that of the exciting light. This phenomenon is called the Stokes shift and allows the detection of emission against a background of light derived from excitation. Usually, the fluorescence excitation spectrum of a fluorophore in a diluted solution is identical to its absorption spectrum and under the same conditions, the fluorescence emission spectrum is independent of the excitation wavelength.

In a diluted solution, fluorescence intensity is linearly proportional to several parameters as deduced from Lambert-Beer’s law. These are the molar absorption coefficient, the path length, the intensity of the incident light, and the quantum yield which is the ratio of the number of emitted to the total number of absorbed photons. Fluorescence detection is dependent on the sensitivity of the instrument and is therefore measured in arbitrary units. Higher concentrations of the fluorophore (> 0.1 absorption units) lead to deviations from the linearity due to loss of excitation intensity across the cuvette path length as the excitation light is absorbed by the fluorophore. This phenomenon is known as the inner filter effect. Other effects which influence fluorescence measurements are related to intrinsic or background fluorescence originating from sample preparations and buffer contaminants, respectively. To minimize fluorescence derived from contaminants it is recommended to use materials of the highest purity. Fluorescence spectra may also be dependent on the solvent. With some fluorophores such as 2-acetylanthracene or tryptophan a spectral shift to longer wavelengths (bathochromic shift or red shift) is observed in more polar solvents. As mentioned with AMC (please see below) the pH of a solution might also change the fluorescence properties of a fluorophore.

**Fluorescence Quenching**

Any process which decreases the fluorescence intensity of a given substance can be referred to as quenching. Several types of quenching processes can be distinguished. These include collisional and static quenching, as well as fluorescence resonance energy transfer (FRET). Collisional or dynamic quenching can be considered as a reduction in fluorescence intensity due to a collision of the quencher with the fluorophore in the excited state. Upon contact the fluorophore returns to the ground state without light emission. One of the best known collisional quenchers which quenches almost all known fluorophores is molecular oxygen. It is therefore often required to remove dissolved oxygen to obtain reliable measurements. In static quenching a non-fluorescent complex is formed between the quencher and the fluorophore. In contrast to both of these quenching processes, FRET does not require contact of the quencher with the fluorophore. The energy transfer occurs without the appearance of a photon.

**Fluorescence Resonance Energy Transfer (FRET)**

Fluorescence resonance energy transfer (FRET) is the transfer of the excited state energy of a donor to an acceptor without the emission of light. The energy transfer can be considered as an energy exchange of an oscillating dipole to a dipole with similar resonance frequency. FRET can only take place when the emission spectrum of the donor overlaps with the absorption spectrum of the acceptor. The donor and acceptor have to be within a distance of 1-10 nm. The energy transfer efficiency depends on the extent of the overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor, the relative orientation of the donor and acceptor transition dipoles, and the distance \( r \) between donor and acceptor. The energy transfer efficiency decreases exponentially by \( r^6 \). The distance at which the efficiency of energy transfer is reduced by 50 % is a characteristic value for a given donor acceptor pair and is called the Förster distance \( R_0 \).
<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Excitation Wavelength</th>
<th>Emission Wavelength</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abz (2-Aminobenzoyl or Anthraniloyl)</td>
<td>320 nm</td>
<td>420 nm</td>
<td>[1], [2], [3]</td>
</tr>
<tr>
<td>Dansyl (5-(Dimethylamino)naphthalene-1-sulfonyl)</td>
<td>342 nm</td>
<td>562 nm</td>
<td>[5]</td>
</tr>
<tr>
<td>EDANS (5-[(2-Aminoethyl)amino]naphthalene-1-sulfonic acid)</td>
<td>340 nm</td>
<td>490 nm</td>
<td>[6]</td>
</tr>
<tr>
<td>FITC (Fluorescein isothiocyanate)</td>
<td>490 nm</td>
<td>520 nm</td>
<td>[7]</td>
</tr>
<tr>
<td>Lucifer Yellow (6-Amino-2,3-dihydro-1,3-dioxo-2-hydrazinocarbonyl-amino-1H-benz[d,e]isoquinoline-5,8-disulfonic acid)</td>
<td>430 nm</td>
<td>520 nm</td>
<td>[8]</td>
</tr>
<tr>
<td>Mca ([7-Methoxycoumarin-4-yl]acetyl)</td>
<td>325 nm</td>
<td>392 nm</td>
<td>[9]</td>
</tr>
<tr>
<td>Trp (Tryptophan)</td>
<td>280 nm</td>
<td>360 nm</td>
<td>[1]</td>
</tr>
</tbody>
</table>

* the values listed are as reported in the cited literature.

**Abz (2-Aminobenzoyl or Anthraniloyl) Substrates**
Abz substrates are generally used in connection with a number of quenchers (Q) such as Dnp (2,4-dinitrophenyl), EDDnp (N-(2,4-dinitrophenyl)ethylenediamine) or 4-nitro-phenylalanine and 3-nitro-tyrosine. Substrate cleavage can be detected at 420 nm using an excitation wavelength of 320 nm.

**N-Me-Abz (N-Methyl-anthraniloyl) Substrates**
N-Me-Abz substrates are generally used with Dnp as quencher (Q). The fluorescent group is either linked to the amino-terminal amino group or the ε-amino group of a lysine residue. Substrate cleavage can be detected at 440-450 nm using an excitation wavelength of 340-360 nm.

**Dansyl (5-(Dimethylamino)naphthalene-1-sulfonyl) Substrates**
In a few substrates the fluorescent dansyl group serves as donor with 4-nitro-phenylalanine as acceptor (Q). Substrate cleavage can be assayed at 562 nm using excitation at 342 nm. More commonly the Dansyl group is used as a quencher for tryptophan fluorescence.

**EDANS (5-[(2-Aminoethyl)amino]naphthalene-1-sulfonic acid) Substrates**
In these substrates, the fluorescence of the EDANS group is generally quenched by the DABCYL (4-(4-dimethylaminophenylazo)benzoyl) group (Q). The DABCYL group is usually conjugated to the N-terminus and the EDANS group (ε_{285 nm} = 24700 M⁻¹ cm⁻¹) attached to the C-terminus of the peptide substrate.
Substrate cleavage can be detected at 490 nm using an excitation wavelength of 340 nm.

**FITC (Fluorescein isothiocyanate) Substrates**

Only few FITC substrates have been described. The FITC label can be quenched with Dnp (Q). Substrate cleavage can be detected at 520 nm using an excitation wavelength of 490 nm.

**Mca ((7-Methoxycoumarin-4-yl)acetyl) Substrates**

In this kind of substrates Mca is bound to an amino group (usually the N-terminal amino group) of a peptide sequence and quenched by Dnp (Q). The cleaved peptide fragment with the attached Mca group can be detected fluorometrically at 392 nm using an excitation wavelength of 325 nm.

**Lucifer Yellow (6-Amino-2,3-dihydro-1,3-dioxo-2-hydrizinocarbonylamino-1H-benz[d]isoquinoline-5,8-disulfonic acid) Substrates**

Lucifer Yellow can be detected at 520 nm using excitation at 430 nm. It is efficiently quenched by Dabsyl (4-(4-Dimethylaminophenylazo)-benzenesulfonfyl) (Q).

**Trp (Tryptophan) Substrates**

Tryptophan (like tyrosine and phenylalanine) is a fluorescent amino acid which has been used in a variety of substrates with Dnp as a quencher (Q). Substrate cleavage can be detected at 360 nm using an excitation wavelength of 280 nm.
Below you can find a list of common donor acceptor pairs used for the design of FRET enzyme substrates.

<table>
<thead>
<tr>
<th>Donor (Fluorophore)</th>
<th>Acceptor (Quencher)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Abz (2-Aminobenzoyl or Anthraniloyl)</td>
<td>Dnp (2,4-Dinitrophenyl)</td>
<td>[1]</td>
</tr>
<tr>
<td>Abz (2-Aminobenzoyl or Anthraniloyl)</td>
<td>EDDnp (N-(2,4-Dinitrophenyl)ethylenediamine)</td>
<td>[10]</td>
</tr>
<tr>
<td>Abz (2-Aminobenzoyl or Anthraniloyl)</td>
<td>4-Nitro-Phe (4-Nitro-phenylalanine)</td>
<td>[11]</td>
</tr>
<tr>
<td>Abz (2-Aminobenzoyl or Anthraniloyl)</td>
<td>3-Nitro-Tyr (3-Nitro-tyrosine)</td>
<td>[12]</td>
</tr>
<tr>
<td>N-Me-Abz (N-Methyl-anthraniloyl)</td>
<td>Dnp (2,4-Dinitrophenyl)</td>
<td>[4]</td>
</tr>
<tr>
<td>Dansyl (5-(Dimethylamino)naphthalene-1-sulfonyl)</td>
<td>4-Nitro-Phe (4-Nitro-phenylalanine)</td>
<td>[5]</td>
</tr>
<tr>
<td>EDANS (5-[(2-Aminoethyl)amino]-naphthalene-1-sulfonic acid)</td>
<td>DABCYL (4-(4-Dimethylaminophenylazo)benzoyl)</td>
<td>[6]</td>
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<tr>
<td>Trp (Tryptophan)</td>
<td>Dnp (2,4-Dinitrophenyl)</td>
<td>[1]</td>
</tr>
<tr>
<td>Trp (Tryptophan)</td>
<td>4-Nitro-Z (4-Nitro-benzyloxyacarbonyl)</td>
<td>[14]</td>
</tr>
</tbody>
</table>

Table 2: Donor/Acceptor Pairs

References

Cathepsin B carboxydipeptidase specificity analysis using internally quenched fluorescent peptides.  

Serpin-derived peptide substrates for investigating the substrate specificity of human tissue kallikreins hK1 and hK2.  
J. Biol. Chem. 272, 29590-29595 (1997)

Hydrolysis of gamma-epsilon isopeptides by cytosolic transglutaminases and by coagulation factor XIIIa.  
J. Biol. Chem. 272, 10311-10317 (1997)

A high throughput fluorogenic substrate for interstitial collagenase (MMP-1) and gelatinase (MMP-9).  

Novel fluorogenic substrates for assaying retroviral proteases by resonance energy transfer. Science 247, 954-958 (1990)


Substrate and inhibitor profile of BACE (beta-secretase) and comparison with other mammalian aspartic proteases. J. Biol. Chem. 277, 4687-4693 (2002)


[12] K. Breddam and M. Meldal

Fluorometric determination of the quality of FITC conjugates. Virologie 28, 41-43 (1977)


For further details, please see the following literature references

J. Bergmeyer

J. Bergmeyer
Methods of Enzymatic Analysis, 3rd Edition, Vol. II, Samples, Reagents, Assessment of Results

J.R. Lakowicz
Principles of Fluorescence Spectroscopy
Cytomegalovirus (CMV) Protease Substrates

M-2450  Abz-tBu-Gly-tBu-Gly-Asn(Me)-2-Ala-Ser-Ser-Arg-Leu-3-nitro-Tyr-Arg-OH
         Trifluoroacetate salt
Improved fluorogenic substrate for the determination of human cytomegalovirus protease. It displayed a kcat/Km value of 15940 M⁻¹s⁻¹, i.e., more than 60-fold greater than that of the equivalent, non-optimized substrate Abz-Val-Val-Asn-Ala-Ser-Ser-Arg-Leu-3-nitro-Tyr-Arg-OH under identical conditions.
Solubility: 1 mg/ml in water

Furin Substrates

         Trifluoroacetate salt
This internally quenched fluorogenic peptide substrate contains anthranilic acid as fluorescent donor and m-nitro-tyrosine as acceptor (quencher). Its sequence is based on the sequence of hemagglutinin. This substrate is efficiently cleaved by furin, a subtilisin-like eukaryotic serine endo-protease with Km = 3.8 µM and kcat = 29.3 s⁻¹ (kcat/Km = 7710000 M⁻¹s⁻¹). Its kcat/Km value is over 2000-fold higher than that of the commonly used substrate Boc-Arg-Val-Arg-Arg-AMC (I-1645).
Solubility: in water

Galanin Degrading Zn-Metallopeptidase Substrates

M-2365  (Abz-Gly¹)-Galanin (1-10)-Lys(retro-m-nitro-Tyr-H²)
            amid (human)
Abz-Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Lys(retro-m-nitro-Tyr-H)-NH₂
         Trifluoroacetate salt
Substrate for the detection of a galanin degrading 70 kD Zn-metallopeptidase from bovine spinal cord. The value of Km was calculated to 72.1 µM and v_max to 18.2 µM/min.
Solubility: 5 mg/ml in methanol

ACE2 Substrates

M-2660  Abz-Ser-Pro-3-nitro-Tyr-OH
Selective substrate for angiotensin-converting enzyme 2 (ACE2), a novel ACE homolog, which differs in its specificity and physiological role from ACE. The internally quenched fluorescent substrate is potentially useful in applications such as high-throughput screening of ACE2 inhibitors (Km = 23 µM, kcat/Km = 3.5 · 10⁴ M⁻¹s⁻¹).
Solubility: at least 1 mg/ml in DMSO or 20 % acetic acid

ACE Substrates

M-1100  Abz-Gly-p-nitro-Phe-Pro-OH
         Trifluoroacetate salt
Fluorogenic substrate for angiotensin I-converting enzyme. 
Solubility: 50 mg/ml in methanol

M-2590  Abz-Phe-Arg-Lys(Dnp)-Pro-OH
         Hydrochloride salt
Excellent angiotensin I-converting enzyme (ACE) substrate with a Km value of 4.0 µM and a kcat value of 210 s⁻¹.
Solubility: in 0.1 % TFA in acetonitrile/water

Cathepsin Substrates

M-2595  Abz-Gly-Ile-Val-Arg-Ala-Lys(Dnp)-OH
Very efficient and selective FRET substrate for human cathepsin B (Km = 5.9 µM, kcat = 43 s⁻¹, kcat/Km = 7288 mM⁻¹s⁻¹). The kcat/Km values for cathepsin K, L, V, X, and cruzain were 133.3, 100, 32, 17, and 75 mM⁻¹s⁻¹, respectively.
Solubility: 1 mg/ml in 50 % acetic acid

M-2600  Abz-Glu-Ile-Phe-Val-Phe-Lys-Gln-EDDnp
         (Abz-EIFVFKQ-EDDnp)
This fluorescence resonance energy transfer (FRET) peptide is a useful substrate for cathepsin P, a recently discovered placental cysteine protease that is structurally related to the more ubiquitously expressed broad-specificity enzyme cathepsin L.
Solubility: 1 mg/ml in 80 % acetic acid
### Human Rhinovirus-14 (HRV14) 2A Protease Substrates

**H-2992** Anthranilyl-HIV Protease Substrate
Abz-Thr-Ile-Nle-p-nitro-Phe-Gln-Arg-NH₂
Trifluoroacetate salt
This fluorogenic hexapeptide substrate is derived from the p24/p15 cleavage site of the viral gag-pol poly-protein. A simple, continuous fluorometric assay for HIV protease has been developed, which allows the screening of potential HIV protease inhibitors.
Solubility: at least 1 mg/ml in DMSO or 20 % acetic acid with agitation

**M-2360** H-Thr-Pro-Ile-Ile-Thr-m-nitro-Tyr-Gly-Pro-Ser-Asp-Lys(Abz)-Tyr-OH
Trifluoroacetate salt
This fluorogenic substrate containing an anthraniloyl group and a 3-nitrotyrosine as the resonance energy transfer donor/quencher pair, was developed for the assay of 2A protease from human rhinovirus (kcat/Km = 154 M⁻¹s⁻¹), which has been viewed as an important enzyme target for antiviral intervention.
Solubility: at least 1 mg/ml in 0.1 % TFA with agitation

### Human Rhinovirus-14 (HRV14) 3C Protease Substrates

**H-1044** Anthranilyl-HIV Protease Substrate III
Abz-His-Lys-Ala-Arg-Val-Val-p-nitro-Phe-Glu-Ala-Nle-Ser-NH₂
Trifluoroacetate salt
Solubility: in water

**H-1052** Anthranilyl-HIV Protease Substrate IV
Abz-Lys-Ala-Arg-Val-Val-p-nitro-Phe-Glu-Ala-Nle-NH₂
Trifluoroacetate salt
Solubility: at least 1 mg/ml in 0.1 % TFA with agitation

**H-1168** Anthranilyl-HIV Protease Substrate V
Abz-Ala-Arg-Val-Val-p-nitro-Phe-Glu-Ala-Nle-NH₂
Trifluoroacetate salt
Solubility: at least 1 mg/ml in DMSO with agitation

**H-1204** Anthranilyl-HIV Protease Substrate VI
Abz-Arg-Val-Val-Val-p-nitro-Phe-Glu-Ala-Nle-NH₂
Trifluoroacetate salt
Hydrolysis of the anthranilyl fluorogenic substrates is monitored by the decrease in fluorescence quenching upon separation of the anthranilyl chromophore from the p-nitro-Phe quencher.
Solubility: in methanol

### Kallikrein Substrates

**M-2665** Abz-Ala-Arg-Phe-Ser-Gln-EDDnp
Trifluoroacetate salt
Best human kallikrein 6 (hK6) FRET substrate so far described (kcat = 11.6 s⁻¹, Km = 0.3 µM, kcat/Km = 38667 mM⁻¹s⁻¹).
Solubility: in 0.1 % TFA in acetonitrile/water

### Papain Substrates

**M-2100** Abz-Gln-Val-Val-Ala-Gly-Ala-EDDnp
Trifluoroacetate salt
This fluorogenic substrate of papain, based on the highly conserved sequence QVVAG of the cystatin family of natural inhibitors, is among the most sensitive papain substrates ever reported (kcat/Km = 29 · 10⁸ M⁻¹s⁻¹).
Lit. C. Serveau et al., Biochimie 76, 153 (1994)
Solubility: 1 mg/ml in 80 % acetic acid

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**Abz/Q** - Substrates (continued)

<table>
<thead>
<tr>
<th>Prod.No.</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2992</td>
<td>Anthranilyl-HIV Protease Substrate</td>
</tr>
<tr>
<td>M-2360</td>
<td>H-Thr-Pro-Ile-Ile-Thr-m-nitro-Tyr-Gly-Pro-Ser-Asp-Lys(Abz)-Tyr-OH</td>
</tr>
<tr>
<td>H-1044</td>
<td>Anthranilyl-HIV Protease Substrate III</td>
</tr>
<tr>
<td>M-2075</td>
<td>Abz-Glu-Thr-Leu-Phe-Gln-Gly-Pro-Val-p-nitro-Phe-NH₂</td>
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<tr>
<td>H-1052</td>
<td>Anthranilyl-HIV Protease Substrate IV</td>
</tr>
<tr>
<td>H-1168</td>
<td>Anthranilyl-HIV Protease Substrate V</td>
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<tr>
<td>H-1204</td>
<td>Anthranilyl-HIV Protease Substrate VI</td>
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<tr>
<td>M-2665</td>
<td>Abz-Ala-Arg-Phe-Ser-Gln-EDDnp</td>
</tr>
<tr>
<td>M-2100</td>
<td>Abz-Gln-Val-Val-Ala-Gly-Ala-EDDnp</td>
</tr>
</tbody>
</table>

* Q = Dnp, EDDnp, p-nitro-Phe, m-nitro-Tyr, p-Nitrobenzylamide
β-Secretase Substrates

M-2560 Abz-Amyloid β/A4 Protein Precursor770 (669-674)-EDDnp
Abz-Val-Lys-Met-Asp-Ala-Glu-EDDnp
(JMV2235; Abz-APP770 (669-674)-EDDnp)

Trifluoroacetate salt
Novel intramolecularly quenched fluorescent substrate containing the Abz/EDDnp groups as the donor/acceptor pair. It mimics the wild-type (JMV2235) β-amyloid precursor protein (βAPP) sequence targeted by β-secretase BACE (β-site APP-cleaving activity). This substrate is cleaved by BACE1, BACE2, and cathepsin D.
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

M-2565 Abz-(Asn670,Leu671)-Amyloid β/A4 Protein Precursor770 (669-674)-EDDnp
Abz-Val-Asn-Leu-Asp-Ala-Glu-EDDnp
(JMV2236; Abz-(Asn670,Leu671)-APP770 (669-674)-EDDnp)

Trifluoroacetate salt
Novel intramolecularly quenched fluorescent substrate containing the Abz/EDDnp groups as the donor/acceptor pair. It corresponds to the Swedish-mutated (JMV2236) β-amyloid precursor (BAPP) sequence targeted by β-secretase BACE (β-site APP-cleaving activity). This substrate is more selectively cleaved by BACE1 and BACE2 than by cathepsin D, a disintegrin and metalloprotease 10 (ADAM10), tumor necrosis factor converting enzyme (TACE), presenilin-1 (PS1), or presenilin-2 (PS2).
Solubility: at least 1 mg/ml in DMSO with agitation

γ-Secretase Substrates

M-2540 Abz-Amyloid β/A4 Protein Precursor770 (708-715)-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide
Abz-Gly-Gly-Val-Ile-Ala-Thr-Val-Lys(Dnp)-D-Arg-D-Arg-D-Arg-NH2
(Asp-APP770 (708-715)-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide; Abz-Amyloid β-Protein (37-42)-Thr-Val-Lys(Dnp)-D-Arg-D-Arg amide)

Trifluoroacetate salt
Novel sensitive fluorogenic substrate developed for the analysis of γ-secretase from post mortem non-Alzheimer’s and Alzheimer’s disease human brain isolates.
Solubility: at least 1 mg/ml in water with agitation

Miscellaneous Substrates

H-6675 Abz-Ala-Gly-Leu-Ala-p-nitrobenzylamide
A fluorogenic substrate for neutral metalloendopeptidases, e.g. Pseudomonas aeruginosa elastase, enkephalinase (NEP 24.11), and thermolysin.
Solubility: 10 mg/ml in 50 % acetic acid

M-2475 Abz-Ala-Phe-Ala-Phe-Asp-Val-Phe-3-nitro-Tyr-Asp-OH
Trifluoroacetate salt
Fluorometric substrate for Asp-specific proteases from Staphylococcus aureus, Bacillus licheniformis and Streptomyces griseus.
Solubility: at least 1 mg/ml in 5 % sodium hydrogen carbonate with agitation

M-2480 Abz-Gly-Ala-Ala-Pro-Phe-3-nitro-Tyr-Asp-OH
Trifluoroacetate salt
Fluorometric substrate for Pro-specific endopeptidases.
Solubility: at least 1 mg/ml in methanol with agitation

H-2638 Abz-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH2
Trifluoroacetate salt
Solubility: 10 mg/ml in water
**N-Me-Abz/Dnp Substrates**

<table>
<thead>
<tr>
<th>Prod.No.</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMP Substrates</strong></td>
<td></td>
</tr>
<tr>
<td>M-1910</td>
<td>Dnp-Pro-β-cyclohexyl-Ala-Abu-Cys(Me)-His-Ala-Lys(N-Me-Abz)-NH₂</td>
</tr>
<tr>
<td></td>
<td>An improved collagenase substrate with a better kcat/Km ratio than substrate M-1855. The Dnp group and the C-terminal N-methyl-anthraniloyl moiety are fluorescence self-quenching until peptide cleavage occurs.</td>
</tr>
<tr>
<td></td>
<td>Solubility: in 50 % acetic acid</td>
</tr>
<tr>
<td>M-2055</td>
<td>Dnp-Pro-β-cyclohexyl-Ala-Gly-Cys(Me)-His-Ala-Lys(N-Me-Abz)-NH₂</td>
</tr>
<tr>
<td></td>
<td>Efficient fluorogenic substrate for two matrix metalloproteinases: interstitial collagenase (MMP-1) and gelatinase (MMP-9). This peptide has favorable solubility characteristics. Both enzymes cleave this substrate between Gly and Cys(Me), liberating a cleavage product with a fluorescence signal suitable for inhibitor screening and determining Ki values. The major advantage of this substrate is its adaptability to filters commonly available on commercial plate readers (excitation at 365 nm and emission at 450 nm).</td>
</tr>
<tr>
<td></td>
<td>Solubility: in 50 % acetic acid</td>
</tr>
<tr>
<td><strong>Dansyl/4-nitro-Phe Substrates</strong></td>
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</tr>
<tr>
<td>Prod.No.</td>
<td>Product</td>
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<td>----------</td>
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<tr>
<td><strong>Neprilysin Substrates</strong></td>
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</tr>
<tr>
<td>M-2650</td>
<td>Dansyl-D-Ala-Gly-4-nitro-Phe-Glu-OH</td>
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<tr>
<td></td>
<td>Trifluoroacetate salt</td>
</tr>
<tr>
<td></td>
<td>Highly sensitive fluorescence resonance energy transfer (FRET) substrate for neutral endopeptidase (NEP or neprilysin EC 3.4.24.11) (Km = 45 µM, kcat = 59 min⁻¹, kcat/Km = 1.3 min⁻¹·µM⁻¹). Enzymatic activity can be monitored with an excitation wavelength of 342 nm and an emission wavelength of 562 nm.</td>
</tr>
<tr>
<td></td>
<td>Solubility: in 0.1 % TFA</td>
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</tr>
<tr>
<td>M-2555</td>
<td>N-Me-Abz-Amyloid β/A4 Protein Precursor (708-715)-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide</td>
</tr>
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<td>N-Me-Abz-Gly-Gly-Val-Ile-Ala-Thr-Val-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide</td>
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<td>N-Me-Abz-APP (37-44)-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide</td>
</tr>
<tr>
<td></td>
<td>Trifluoroacetate salt</td>
</tr>
<tr>
<td></td>
<td>Novel intramolecularly quenched fluorescent, presenilin-dependent substrate for assaying γ-secretase activity. It has been used for partial purification and characterization of γ-secretase from post-mortem human brain.</td>
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<tr>
<td></td>
<td>Solubility: at least 1 mg/ml in water with agitation</td>
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</table>

<table>
<thead>
<tr>
<th>Prod.No.</th>
<th>Product</th>
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<tbody>
<tr>
<td><strong>Miscellaneous Substrates</strong></td>
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</tr>
<tr>
<td>M-2145</td>
<td>N-Me-Abz-Lys-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH₂</td>
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<td></td>
<td>Trifluoroacetate salt</td>
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<tr>
<td></td>
<td>Solubility: 10 mg/ml in water</td>
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</table>
**ADAM Protein Substrates**

**M-2545**  
**Glu(EDANS)-ADAM 8 (165-172)-Lys(DABCYL)amide (human)**

H-Glu(EDANS)-Arg-Thr-Ala-Ala-Val-Phe-Arg-Pro-Lys(DABCYL)-NH₂  
**Glu(EDANS)-CD156a Antigen (165-172)-Lys(DABCYL)amide (human); Glu(EDANS)-Cell Surface Antigen MS2 (165-172)-Lys(DABCYL)amide (human))**

**Trifluoroacetate salt**  
Novel fluorescent peptide substrate for ADAM 28 which is a member of the ADAM family of disintegrin metalloproteases.  
Solubility: at least 1 mg/ml in DMSO or 20 % acetic acid with agitation

**M-2535**  
**H-Glu(EDANS)-Lys-Pro-Ala-Lys-Phe-Phe-Arg-Leu-Lys(DABCYL)-NH₂ Trifluoroacetate salt**

Novel quenched fluorescent substrate for ADAM 8, ADAM 15, and MDC-L (ADAM 28) but not for ADAM 17. For ADAM 8 half maximal cleavage was observed at 2.5 µM.  
Solubility: at least 1 mg/ml in DMSO or 20 % acetic acid with agitation

**Calpain-1 Substrates**

**M-2655**  
**H-Glu(EDANS)-Pro-Leu-Phe-Ala-Glu-Arg-Lys(DABCYL)-OH**

Internally quenched substrate for calpain-1 (µ-calpain) with optimal cleavage motifs flanking the scissile bond. The enzyme showed a more than 18-fold higher turnover rate for the hydrolysis of this FRET substrate based on the amino acid sequence PLFAER than for EVYGMM a sequence derived from the cleavage site of the natural substrate α-spectrin.  
Lit. D. Cuerrier et al., J. Biol. Chem. 280, 40632 (2005)  
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

**Caspase-1 Substrates**

**M-1940**  
**DABCYL-Tyr-Val-Ala-Asp-Ala-Val-EDANS**

**(DABCYL-YVADPV-EDANS)**

**Trifluoroacetate salt**

This fluorogenic caspase-1 (ICE) substrate is based on the principle of resonance energy transfer. It allows a continuous assay of caspase-1 that is useful in the screening of inhibitory compounds (Km = 11.4 µM, kcat = 0.79 s⁻¹).  
Solubility: at least 1 mg/ml in 50 % acetic acid

**Cathepsin Substrates**

**M-2295**  
**Ac-Glu-Asp(EDANS)-Lys-Pro-Ile-Leu-Phe-Arg-Leu-Gly-Lys(DABCYL)-Glu-NH₂ Trifluoroacetate salt**

Sensitive fluorescent peptide substrate for cathepsin D, an enzyme, that can degrade extracellular matrix components and may facilitate the spread of tumor cells. High levels of active cathepsin D were found within senile plaques in brains of Alzheimer’s patients.  
Lit. S.V. Guzik et al., FEBS Lett. 413, 379 (1997)  
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

**Cytomegalovirus (CMV) Protease Substrates**

**M-2060**  
**Fluorogenic Human CMV Protease Substrate**

DABCYL-Arg-Gly-Val-Val-Asn-Ala-Ser-Ser-Arg-Leu-Ala-EDANS**

**Trifluoroacetate salt**

This substrate has been synthesized to develop a fluorescence-based assay of human cytomegalovirus proteinase. It is cleaved specifically at the Ala-Ser bond thereby liberating the C-terminal peptide-EDANS fragment from the proximity quenching effect of the DABCYL group. This represents the first fluorescence-based assay of the herpes virus proteases and permits the characterization of potential inhibitors.  
Solubility: at least 1 mg/ml in 20 % acetic acid with agitation

**HCV NS3 Protease Substrates**

**M-2235**  
**Ac-Asp-Glu(EDANS)-Glu-Glu-Alb-L-lactoyl-Ser-Lys(DABCYL)-NH₂ Trifluoroacetate salt**

Internally quenched fluorogenic substrate for the continuous monitoring of HCV NS3 protease activity.  
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

**EDANS/DABCYL Substrates**
EDANS/DABCYL Substrates (continued)

Hepatitis A Virus (HAV) 3C Protease Substrates

M-1900
Hepatitis A Virus (HAV) 3C Protease Substrate
DABCYL-Gly-Leu-Arg-Thr-Gln-Ser-Phe-Ser-EDANS
Trifluoroacetate salt
This fluorogenic substrate represents a cleavage sequence specific for the picornavirus 3C protease. Thus, it has been found to be highly sensitive for the 3C protease from hepatitis A virus (HAV).
Solubility: at least 1 mg/ml in 50 % acetic acid with agitation

HIV Protease Substrates

M-1865
DABCYL-γ-Abu-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-EDANS
Trifluoroacetate salt
This fluorogenic HIV-1 protease substrate consists of an octapeptide with a fluorescent donor (EDANS) and a quenching acceptor (DABCYL), attached at the COOH- and NH₂-termini. The γ-Abu spacer was inserted to avoid potential steric hindrance of substrate binding by the bulky acceptor. This substrate is cleaved by the HIV-1 protease at the Tyr-Pro bond which results in a time-dependent increase in fluorescence intensity.
Solubility: at least 1 mg/ml in DMSO or 80 % acetic acid with agitation

Kaposi’s Sarcoma-Associated Herpes Virus (KSHV) Protease Substrates

M-2355
H-Glu(EDANS)-Val-Tyr-Leu-Lys-Ala-Ser-Gln-Phe-Pro-Ala-Gly-Ile-Lys(DABCYL)-Gly-OH
Trifluoroacetate salt
KSHV release site (R-site) substrate with a kcat/Km = 0.52 x 10² M⁻¹min⁻¹. 
Solubility: at least 1 mg/ml in 20 % acetic acid with agitation

Malaria Aspartyl Proteinase Substrates

M-2120
DABCYL-Glu-Arg-Nle-Phe-Leu-Ser-Phe-Pro-EDANS
Trifluoroacetate salt
Useful peptide substrate for a continuous fluorescence-based assay of the malaria aspartyl proteinase. The peptide sequence is derived from the cleavage site present in hemoglobin, with Nle as a substitution for Met to avoid potential oxidation related problems.
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

MMP Substrates

M-2490
DABCYL-γ-Abu-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Glu(EDANS)-Ala-Lys-NH₂
A selective fluorogenic matrix metalloproteinase (MMP) substrate. The kcat/Km value for MMP-3 (Stromelysin-1) is 29000 M⁻¹s⁻¹, for MMP-13 13000 M⁻¹s⁻¹, and for MMP-9 7500 M⁻¹s⁻¹ (at pH 7.5, 25 °C). The fluorescence increase is monitored using an excitation wavelength of 360 nm and an emission wavelength of 490 nm. No activity against this substrate could be measured with MMP1, MMP-7, and MMP-8 (kcat/Km < 1000 M⁻¹s⁻¹).
Lit. B. Beekman et al., FEBS Lett. 418, 305 (1997)
Solubility: in water

M-2495
DABCYL-γ-Abu-Pro-Gln-Gly-Leu-Glu(EDANS)-Ala-Lys-NH₂
Highly soluble fluorogenic matrix metalloproteinase (MMP) substrate with the following kcat/Km values (at pH 7.6, 37 °C): 29000 M⁻¹s⁻¹ for MMP-3, 209000 M⁻¹s⁻¹ for MMP-9, 40000 M⁻¹s⁻¹ for MMP-3, and 21000 M⁻¹s⁻¹ for MMP-1. The fluorescence increase is measured using an excitation wavelength of 360 nm and an emission wavelength of 480 nm. Due to the relatively high emission wavelength of EDANS the substrate allows convenient measurement of MMP activity in complex biological media like synovial fluid and culture medium.
Solubility: 1 mg/ml in water
### Renin Substrates

**M-2050**  
DABCYL-γ-Abu-Ile-His-Pro-Phe-Leu-Val-Ile-His-Thr-EDANS  
**Trifluoroacetate salt**  
This fluorogenic peptide substrate has been developed to continuously measure the proteolytic activity of human renin (Km = 1.5 µM at physiological pH). Cleavage of the substrate occurs specifically at the Leu-Val bond and corresponds to the renin cleavage site of angiotensinogen. By means of this assay as low as 30 ng/ml renin can be detected after an incubation of only 3-5 min.  
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

### SARS Main Protease Substrates

**M-2575**  
DABCYL-Lys-HCoV-SARS Replicase Polyprotein 1ab (3235-3246)-Glu-EDANS  
DABCYL-Lys-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-Met-Glu-EDANS (DABCYL-Lys-pp1ab (3235-3246)-Glu-EDANS (Human coronavirus) (strain SARS); DABCYL-Lys-ORF1AB (3235-3246)-Glu-EDANS (Human coronavirus) (strain SARS))  
**Trifluoroacetate salt**  
Sensitive internally quenched fluorogenic substrate for SARS main protease with a Km value of 17 µM and a kcat value of 1.9 s⁻¹.  
Solubility: 1 mg/ml in 50 % acetic acid

### β-Secretase Substrates

**M-2445**  
DABCYL-(Asn⁴⁷⁹,Leu⁴⁸⁷)-Amyloid β/A4 Protein Precursor, 770 (661-675)-EDANS  
DABCYL-Ile-Lys-Thr-Glu-Glu-Ile-Leu-Asp-Ala-Glu-Phe-EDANS (DABCYL-(Asn⁴⁷⁹,Leu⁴⁸⁷)-APP, 770 (661-675)-EDANS)  
**Ammonium salt**  
Solubility: 1 mg/ml in TFA

**M-2435**  
DABCYL-(Asn⁴⁷⁹,Leu⁴⁸⁷)-Amyloid β/A4 Protein Precursor, 770 (667-675)-EDANS  
DABCYL-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-EDANS (DABCYL-(Asn⁴⁷⁹,Leu⁴⁸⁷)-APP, 770 (667-675)-EDANS)  
**Ammonium salt**  
Solubility: 1 mg/ml in water

### Miscellaneous Substrates

**M-2380**  
DABCYL-[Nle²⁰²⁷]-Collagen Type III α1 chain (1062-1069)-EDANS (mouse)  
DABCYL-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Nle-EDANS (DABCYL-(Asp¹⁰⁸⁷,Nle¹⁰⁹⁷)-Collagen Type III α1 chain (1062-1069)-EDANS (mouse))  
Substrate for metalloproteinases.  
Solubility: 1 mg/ml in methanol

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**FRET Substrates 13**
**FITC/Dnp Substrates**

**Caspase-1 Substrates**

M-2285  FITC-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-OH  
(Contains FITC isomer I)  
(FITC-YVADAPK(Dnp))  
Specific substrate for the determination of caspase-1 and caspase-1 like enzyme activities. Cleavage of this peptide substrate at the P1 Asp residue results in a continuous fluorescent assay. It is useful both in FACS and fluorescence microscopy experiments. Caspase-3 is only weakly active using this substrate. See also our reference substance M-2280.  
Solubility: in basic solvents

**Lucifer Yellow/Dabsyl Substrates**

**β-Secretase Substrates**

M-2570  Lys(Dabsyl)-(Asn<sup>670</sup>,Leu<sup>671</sup>)-Amyloid β/A4 Protein Precursor<sub>770</sub> (667-676)-Gln-Lucifer Yellow  
H-Lys(Dabsyl)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Gln-Lucifer Yellow  
(Lys(Dabsyl)-(Asn<sup>670</sup>,Leu<sup>671</sup>)-APP<sub>770</sub> (667-676)-Gln-Lucifer Yellow)  
**Ammonium salt**  
A highly selective substrate for measuring BACE1 (K<sub>m</sub> = 9 µM, k<sub>cat</sub> = 0.02 s<sup>-1</sup>) and BACE2 activity. In this fluorescence resonance energy transfer (FRET) substrate the fluorescent group Lucifer Yellow is efficiently quenched by Dabsyl (4-(4-Dimethylaminophenylazo)benzenesulfonyl). Enzymatic cleavage can be assayed by detecting the increase in fluorescence emission at 520 nm using an excitation wavelength of 430 nm.  
Solubility: 1 mg/ml in 1 N ammonia
Mca/Dnp Substrates

Caspase-1 Substrates

M-2195  Mca-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-OH
(Mca-YVADAPK(Dnp))
Trifluoroacetate salt
Specific highly fluorescent substrate for the determination of caspase-1 (ICE) and caspase-1-like enzyme activities. Cleavage of this peptide substrate at the P1 Asp residue results in a continuous fluorescent assay monitored at an emission wavelength of 392 nm. Caspase-3 (apopain, CPP-32) is only weakly active on this substrate. Both caspase-1 and caspase-3 are involved in apoptosis.
Solubility: at least 1 mg/ml in 80 % acetic acid or DMSO with agitation

Caspase-3 Substrates

M-2200  Mca-Asp-Glu-Val-Asp-Ala-Pro-Lys(Dnp)-OH
(Mca-DEVADAPK(Dnp))
Trifluoroacetate salt
Specific highly fluorescent substrate for the determination of caspase-3 (also named apopain or CPP-32) and CPP-32-like enzyme activities. Cleavage of this peptide substrate at the P1 Asp residue results in a continuous fluorescent assay monitored at an emission wavelength of 392 nm. Caspase-1 has only little activity on this substrate.
Solubility: at least 1 mg/ml in 0.1 M sodium hydrogen carbonate with agitation

Caspase-4 Substrates

M-2315  Mca-Leu-Glu-Val-Asp-Gly-Trp-Lys(Dnp)-NH₂
(Mca-LEVGDWK(Dnp)-NH₂)
This fluorogenic resonance energy transfer substrate for caspase-4 (ICH-2) exhibits an excitation wavelength of 325 nm and an emission wavelength of 392 nm.
Lit. R.V. Talanian et al., J. Biol. Chem. 272, 9677 (1997)
Solubility: in TFA and DMSO

Cathepsin Substrates

M-2455  Mca-Gly-Lys-Pro-Ile-Leu-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH₂
Trifluoroacetate salt
Substrate for cathepsin D and E.
Lit. Y. Yasuda et al., J. Biochem. 125, 1137 (1999)
Solubility: 1 mg/ml in 50 % acetic acid

M-2625  Mca-Gly-Ser-Pro-Ala-Phe-Leu-Ala-Lys(Dnp)-D-Arg-NH₂
Trifluoroacetate salt
Highly sensitive and selective cathepsin E FRET substrate derived from the cleavage site sequence of human ß2-macroglobulin (Km = 1.9 µM; kcat/Km = 10.2 µM s⁻¹ for human erythrocyte cathepsin E). The substrate was resistant to hydrolysis by the analogous aspartic proteinases cathepsin D and pepsin, as well as the lysosomal cysteine proteinases cathepsin B, L, and H. Useful for monitoring and accurately quantifying cathepsin E, even in crude enzyme preparations (excitation wavelength at 328 nm, emission wavelength at 393 nm).
Solubility: in 0.1 % TFA in acetonitrile/water

Endothelin-Converting Enzyme-1 Substrates

M-2405  Mca-(Ala₇,Lys(Dnp)₉)-Bradykinin
Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(Dnp)-OH
Trifluoroacetate salt
Very sensitive internally quenched fluorescent substrate for endothelin-converting enzyme-1 (ECE-1) a membrane-bound zinc metallopeptidase that is related to neprilysin in amino acid sequence. The kcat/Km value for the hydrolysis by ECE-1 was 1.9 ∙ 10⁻³ M⁻¹s⁻¹ and 1.7 ∙ 10⁻³ M⁻¹s⁻¹ for the hydrolysis by neprilysin. For MMP-2 and MMP-9 the kcat/Km values are in the range of 10⁻³ M⁻¹s⁻¹ whereas for MMP-1 there was no hydrolysis observed.
Solubility: 1 mg/ml in 80 % acetic acid

Human Herpes Virus 8 (HHV-8) Protease Substrates

M-2260  Mca-γ-Abu-Asn-Arg-Leu-Glu-ALA-Ser-Arg-Ser-Ser-Lys(Dnp)-NH₂
Trifluoroacetate salt
Fluorogenic substrate containing the M-site of Kaposi’s sarcoma-associated herpes virus (KSHV) (also called human herpes virus 8 (HHV-8)) for the assay of KSHV protease (kcat/Km = 165 M⁻¹s⁻¹). Time-evolved fluorescence enhancement was monitored by exciting this KSHV M-site peptide at 325 nm and detecting emission at 393 nm.
Lit. A. Uenal et al., J. Virol. 71, 7030 (1997)
Solubility: at least 1 mg/ml in 80 % acetic acid with agitation
### Mca/Dnp Substrates (continued)

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<tr>
<th>Prod.No.</th>
<th>Product</th>
<th>Description</th>
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<td><strong>MMP Substrates</strong></td>
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<tr>
<td><strong>M-2105</strong></td>
<td>Mca-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Trifluoroacetate salt</td>
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<td>This substrate was hydrolyzed 60 times more rapidly by stromelysin 1 (MMP-3) (kcat/Km = 59400 M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;) than by interstitial collagenase (MMP-1). However it showed little discrimination between MMP-3, gelatinase A (MMP-2) (kcat/Km = 54000 M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;), and gelatinase B (MMP-9) (kcat/Km = 55300 M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;).</td>
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<td>Solubility: at least 1 mg/ml in 50 % acetic acid</td>
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<td><strong>M-2110</strong></td>
<td>Mca-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Trifluoroacetate salt</td>
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<td>This substrate was hydrolyzed rapidly by stromelysin 1 (MMP-3) with kcat/Km = 218000 M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt; and very slowly by gelatinase B (MMP-9) (kcat/Km = 10100 M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;). There was no hydrolysis by MMP-1 and MMP-2.</td>
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<td>Solubility: at least 1 mg/ml in water with agitation</td>
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<td><strong>M-2350</strong></td>
<td>Mca-Lys-Pro-Glu-Leu-Dap(Dnp)-Ala-Arg-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Trifluoroacetate salt</td>
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<td>The N-terminal elongation of the widely used MMP substrate Mca-Pro-Leu-Glu-Dap(Dnp)-Ala-Arg-NH&lt;sub&gt;2&lt;/sub&gt; (M-1895) with a Lys yields a fluorogenic peptide with improved substrate properties. As compared to M-1895, the specificity constant (kcat/Km) of M-2350 for collagenases (MMP-1, MMP-8, MMP-13) and MMP-14 is increased two- to ninefold and threefold, respectively, while those for gelatinases and matrilysin remain equally high.</td>
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<td>Solubility: 1 mg/ml in 0.5 % ammonium hydroxide</td>
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<td><strong>M-2510</strong></td>
<td>Mca-Pro-Leu-Ala-Cys(Mob)-Trp-Ala-Arg-Dap(Dnp)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Trifluoroacetate salt</td>
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<td>Fluorogenic substrate for MMP-14 (MT1-MMP) and stromelysin-3 (ST3). It displayed a kcat/Km value of 3.6 · 10&lt;sup&gt;4&lt;/sup&gt; M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt; and 7.3 · 10&lt;sup&gt;5&lt;/sup&gt; M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;, when assayed with ST3 and MMP-14, respectively.</td>
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<td>Solubility: 5 mg/ml in 50 % acetic acid</td>
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<td><strong>M-2520</strong></td>
<td>Mca-Pro-Leu-Ala-Nva-Dap(Dnp)-Ala-Arg-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Trifluoroacetate salt</td>
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<td>Among the fluorescent peptide substrates analyzed, Mca-Pro-Leu-Ala-Nva-Dap(Dnp)-Ala-Arg-NH&lt;sub&gt;2&lt;/sub&gt; displayed the highest specificity constant with MMP-26 (kcat/Km = 3.0 · 10&lt;sup&gt;-5&lt;/sup&gt; M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;, pH 7.5, T = 25 °C). The fluorescence was measured at an excitation wavelength of 328 nm and emission wavelength of 393 nm.</td>
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<td>Solubility in TFA buffer</td>
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<td><strong>M-2670</strong></td>
<td>Mca-Pro-Leu-Glu-Glu-Ala-Dap(Dnp)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Trifluoroacetate salt</td>
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<td>Highly selective MMP-12 FRET substrate with a kcat/Km value of 1.85 · 10&lt;sup&gt;-4&lt;/sup&gt; M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;. Poor substrate of other MMPs with the exception of MMP-13 (kcat/Km = 0.53 · 10&lt;sup&gt;-5&lt;/sup&gt; M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;) and MMP-9 (0.33 · 10&lt;sup&gt;-5&lt;/sup&gt; M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;).</td>
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<td>Solubility: 10 mg/ml in 50 % acetic acid</td>
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<td><strong>β-Secretase Substrates</strong></td>
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<td><strong>M-2425</strong></td>
<td>Mca-(Asn&lt;sup&gt;667&lt;/sup&gt;-Leu&lt;sup&gt;671&lt;/sup&gt;)-Amyloid β/A4 Protein Precursor&lt;sub&gt;770&lt;/sub&gt;</td>
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<td>Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Dap(Dnp)-OH (Mca-(Asn&lt;sup&gt;667&lt;/sup&gt;-Leu&lt;sup&gt;671&lt;/sup&gt;)-APP&lt;sub&gt;770&lt;/sub&gt; (667-674)-Dap(Dnp))</td>
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<td>Ammonium acetate salt</td>
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<tr>
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<td>Solubility: 1 mg/ml in TFA or 0.1 mg/ml in water</td>
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<tr>
<td><strong>M-2420</strong></td>
<td>Mca-(Asn&lt;sup&gt;667&lt;/sup&gt;-Leu&lt;sup&gt;671&lt;/sup&gt;)-Amyloid β/A4 Protein Precursor&lt;sub&gt;770&lt;/sub&gt;</td>
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<td>Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(Dnp)-OH (Mca-(Asn&lt;sup&gt;667&lt;/sup&gt;-Leu&lt;sup&gt;671&lt;/sup&gt;)-APP&lt;sub&gt;770&lt;/sub&gt; (667-675)-Lys(Dnp))</td>
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<td>Ammonium acetate salt</td>
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<td>Solubility: Fluorogenic substrate for pro-memapsin-2 containing the β-secretase site of the Swedish mutation of APP</td>
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<td>Solubility: 1 mg/ml in 0.5 % ammonium hydroxide</td>
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**Prod.No.**  Product
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**M-2485**

Mca-(Asn<sup>670</sup>,Leu<sup>671</sup>)-Amyloid β/A4 Protein Precursor<sub>770</sub> (667-675)-Lys(Dnp) amide

Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(Dnp)-NH<sub>2</sub> (Mca-(Asn<sup>670</sup>,Leu<sup>671</sup>)-APP<sub>770</sub> (667-675)-Lys(Dnp) amide)

Trifluoroacetate salt

Fluorogenic substrate for pro-memapsin-2 containing the β-secretase site of the Swedish mutation of APP. The kinetic parameters at pH 4.5 are \(K_m = 4.5 \mu M\) and \(k_{cat} = 0.25 \text{ min}^{-1}\). Lit. J. Ermolieff et al., Biochemistry 39, 16263 (2000)/ J. Ermolieff et al., Biochemistry 39, 12450 (2000)

Solubility: at least 1 mg/ml in DMSO with agitation

**M-2460**

Mca-Amyloid β/A4 Protein Precursor<sub>770</sub> (667-676)-Lys(Dnp)-Arg-Arg amide

Mca-Ser-Glu-Val-Lys-Met-Asp-Ala-Glu-Phe-Arg-Lys(Dnp)-Arg-Arg-NH<sub>2</sub> (Mca-APP<sub>770</sub> (667-676)-Lys(Dnp)-Arg-Arg amide)

Trifluoroacetate salt

This fluorescent peptide substrate contains the wild-type amyloid precursor protein (APP) β-secretase cleavage site. It has been used for assaying β-secretase-like activity of thimet oligopeptidase (TOP, EC 3.4.24.15). The results suggested that TOP is a potential β-secretase candidate and is involved in the processing of APP in vivo.

Lit. H. Koike et al., J. Biochem. 126, 235 (1999)

Solubility: 1 mg/ml in water

**M-2465**

Mca-(Asn<sup>670</sup>,Leu<sup>671</sup>)-Amyloid β/A4 Protein Precursor<sub>770</sub> (669-674)-Lys(Dnp)

Mca-Val-Asn-Leu-Asp-Ala-Glu-Lys(Dnp)-OH (Mca-(Asn<sup>670</sup>,Leu<sup>671</sup>)-APP<sub>770</sub> (669-674)-Lys(Dnp))

Trifluoroacetate salt

This fluorescent peptide substrate contains the 'Swedish' Lys-Met/Asn-Leu mutation of the amyloid precursor protein (APP) β-secretase cleavage site. It has been used for assaying β-secretase-like activity of thimet oligopeptidase (TOP, EC 3.4.24.15). The results suggested that TOP is a potential β-secretase candidate and is involved in the processing of APP in vivo.

Lit. H. Koike et al., J. Biochem. 126, 235 (1999)

Solubility: 1 mg/ml in 50 % TFA

**M-2440**

Mca-(Asn<sup>670</sup>,Leu<sup>671</sup>)-Amyloid β/A4 Protein Precursor<sub>770</sub> (669-674)-Lys(Dnp)

Mca-Val-Asn-Leu-Asp-Ala-Glu-Lys(Dnp)-OH (Mca-(Asn<sup>670</sup>,Leu<sup>671</sup>)-APP<sub>770</sub> (669-674)-Lys(Dnp))

Solubility: 10 mg/ml in 1 % ammonium hydroxide

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**Thimet Oligopeptidase Substrates**

**M-2270**

Mca-Pro-Leu-Gly-Pro-D-Lys(Dnp)-OH

Specific highly fluorescent substrate for the determination of the thimet oligopeptidase (EP 24.15, EC 3.4.24.15) activity.


Solubility: 10 mg/ml in 50 % acetic acid

**TNF-α Converting Enzyme Substrates**

**M-2255**

Mca-(endo-1a-Dap(Dnp))-TNF-α (-5 to +6) amide (human)

Mca-Pro-Leu-Ala-Gln-Ala-Val-Dap(Dnp)-Arg-Ser-Ser-Ser-Arg-NH<sub>2</sub> (Mca-APP<sub>770</sub> (667-676)-Lys(Dnp)-Arg-Arg amide)

Trifluoroacetate salt

Fluorogenic peptide substrate for tumor necrosis factor (TNF) converting enzyme.


Solubility: at least 1 mg/ml in ½ portion of acetonitrile, then ½ portion of water with agitation

**Miscellaneous Substrates**

**M-2390**

Mca-Arg-Pro-Leu-Ala-Leu-Trp-Dap(Dnp)-NH<sub>2</sub>

Trifluoroacetate salt

Solubility: 1 mg/ml in 50 % acetic acid

**M-2225**

Mca-Pro-Lys-Pro-Leu-Ala-Leu-Dap(Dnp)-Ala-Arg-NH<sub>2</sub>

Trifluoroacetate salt


Solubility: at least 1 mg/ml in 0.1 % TFA

**M-2395**

Mca-(Gln<sup>192</sup>)-Succinate Semialdehyde Dehydrogenase (186-192)-Dap(Dnp) amide (human, E. coli)

Mca-Thr-Pro-Phe-Ser-Ala-Leu-Gln-Dap(Dnp)-NH<sub>2</sub>

Trifluoroacetate salt

Solubility: 1 mg/ml in 50 % acetic acid

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FRET Substrates 17
MMP Substrates

M-2205 Dnp-Arg-Pro-Leu-Leu-Leu-Trp-Arg-Ser-OH
Trifluoroacetate salt
This peptide is the best fluorogenic substrate developed for matrilysin (also called punctuated metalloproteinase 1, PUMP-1 or MMP-7) thus far. In addition to good kinetic parameters ($k_{cat}/K_{m} = 1.9 \cdot 10^2 \text{ M}^{-1}\text{s}^{-1}$), it showed good solubility in assay buffer.
Solubility: at least 1 mg/ml in 10 % acetic acid with agitation

M-1855 Dnp-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH$_2$
Trifluoroacetate salt
This fluorogenic peptide is an efficient substrate for interstitial/vertebrate collagenase (MMP-1), stromelysin (MMP-3), and especially for the gelatinases (MMP-2 and MMP-9) and the punctuated metalloproteinase (MMP-7).
Solubility: at least 1 mg/ml in 50 % acetic acid with agitation

Trp/Dnp Substrates

MMP Substrates

M-1595 4-Nitro-Z-Gly-Trp-Gly-OH
Fluorogenic substrate for a simple, sensitive, and reproducible assay of angiotensin I-converting enzyme, especially in human plasma.
Solubility: 50 mg/ml in methanol
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